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PROVISIONAL SPECIFICATION

for the invention entitled:

"Modified cells and methods of using same"

The invention is described in the following statement:

MODIFIED CELLS AND METHODS OF USING SAME

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention relates generally to a model system to identify haemopoietic cells of particular lineages and their stage of differentiation. More particularly, the present invention provides genetically modified cells and non-human animals comprising such cells which carry a genetic marker of terminal differentiation modified to co-produce a reporter molecule capable of eliciting an identifiable signal. More particularly, the present invention provides a genetically modified cell or an *in vivo* or *in vitro* system comprising cells which co-express genetic material which encodes Blimp and a reporter molecule. Detection of reporter activity in cells of a haematopoietic lineage, such as but not limited to a B-cell lineage, is indicative that cells having reporter activity are committed to differentiation into a cell type such as an antibody secreting cell (ASC). The present invention provides therefore, genetically modified cells or non-human animals comprising such cells which monitor the differentiation or transformation status of cells under various conditions or in the presence of various stimuli or agents. The present invention further provides screening methods including high through-put screening methods for identifying molecules capable of modulating the differentiation or transformation status of cells, such as, without limitation, embryonic cells during development, cells with aberrant differentiation such as cancer cells, and cells of the haematopoietic cell lineages such as, for example B and/or T cells. Identified molecules form the basis for pharmaceutical compositions for therapeutic and prophylactic application.

DESCRIPTION OF THE PRIOR ART

Bibliographic details of references in the subject specification are also listed at the end of the specification.

Reference to any prior art in this specification is not, and should not be taken as, an acknowledgement or any form of suggestion that this prior art forms part of the common general knowledge in any country.

Cellular life involves a myriad of alternative and highly regulated biochemical pathways directing changes in cell division, differentiation, morphogenesis and apoptosis. Cells vary in their potential to divide and/or differentiate. For example, the embryo comprises totipotent cells retaining the ability to differentiate into any cell type. Other cell types including stem cells are pluripotent and may ultimately differentiate into a range of but not all cell phenotypes. Some cells become committed to one final form: they are terminally differentiated.

Changes which block normal maturation of cells into terminally differentiated cells or which prevent apoptosis can act as triggers for tumor development characterized by uncontrolled cell division without differentiation or cell death. Thus, agents which promote differentiation and normal apoptosis may switch off tumor development.

Molecules which are expressed during the time of terminal differentiation of particular cell types have been intensely studied. However, in order to understand the sequence of events during this period at a molecular level it is necessary to understand the temporal and spatial expression patterns of molecules which are expressed in this phase of development.

B lymphocyte-induced maturation factor (Blimp) is a 98kDa transcription factor which was originally identified as being induced during the differentiation of a B-cell lymphoma cell line (Turner *et al., Cell 77*:297, 1994). The corresponding factor from human cells is referred to a PRDM-1. It has been proposed that Blimp-1 has a pre-eminent role in regulating B-cell terminal differentiation. Specifically, *Blimp-1* is expressed in antibody secreting cells (ASC) from man and mouse but it is not expressed in memory cells (Angelin-Duclos *et al., J Immunol 165:5462*, 2000). Ectopic expression of *Blimp-1* is sufficient to drive terminal differentiation of lymphomas and primary B-cells into ASC cells (Turner *et al., (supra)*, Schliephake *et al., Eur J Immunol 26:268*, 1996; Messika *et*

al., J Exp Med 188:515, 1998; Knodel *et al., Eur J Immunol 31:1972*, 2001). Blocking expression of *Blimp-1* through antisense or dominant-interfering approaches suppresses cell-cycle exit which is thought to be essential for full ASC differentiation (Soro *et al., J Immunol 163:611*, 1999; Angelin-Duclos *et al., J Immunol 165:5462*, 2000; Johnson *et al., Eur J Immunol 32:3765*, 2002). Also, mice which lack Blimp-1 in B-cells produce very little immunoglobulin and have a markedly reduced ASC compartment. (Shapiro-Shelef *et al., Immunity 19:607*, 2003.).

It was initially reported that Blimp-1 is only produced in cells of the B-cell lineage, however, it is now evident that Blimp-1 is produced in other cell lineages such as during myeloid differentiation (Keller *et al., Genes Dev 5:868*, 1991, Chang *et al., Nat Immunol 1:169*, 2000). Blimp-1 is required for the repression of *c-myc* which is important during myeloid differentiation (Chang *et al., (supra)*, 2000; Marcu *et al., Annu Rev Biochem 61:809*, 1992). Over production of Blimp-1 in U937 is sufficient to induce macrophage differentiation (Chang *et al., (supra)*, 2000). Thus repression of *c-myc* by Blimp-1 in macrophages and B-cells is a common feature of terminal differentiation in these two lineages.

Blimp-1 is also broadly produced during mouse and *Xenopus* embryonic development (de Souza *et al., Embo J 18:6062*, 1999; Rosenbaum *et al., Embo J, 9:897*, 1990).

B-lymphocytes are among the most intensively studied eukaryotic cell types but while the early steps of B-cell development are relatively well characterized, much less is known about the processes which control the final differentiation of B-lymphocytes into ASC. ASC or plasma cells are the direct mediators of the humoral immune response. They secrete a large amount of serum immunoglobulin essential for protective immunity. The terminal differentiation of B-lymphocytes into ASC is, therefore, a subject of intense therapeutic interest. For example, terminal differentiation to ASC is a crucial element in effective vaccination strategies. Furthermore, multiple myeloma results from the failure of an ASC to complete the differentiation pathway.

However, ASC represent a very rare population of highly specialised cells located mostly in the bone marrow and spleen. ASC populations in mice and man comprise cells of heterogeneous life span and cell surface phenotype making a definitive prospective isolation of pure ASC impossible (Fong *et al.*, *Proc Natl Acad Sci U S A 11:11*, 2003; Medina *et al.*, *Blood 99:2154*, 2002; O'Connor *et al.*, *J. Exp Med 195:737*, 2002; Manz *et al.*, *Curr Opin Immunol 14:517*, 2002; Underhill *et al.*, *Blood 24:24*, 2003).

The ability to monitor terminal differentiation of ASC and other cells of the haemopoietic system in a wide range of contexts and under various stimuli would be extremely valuable in developing strategies and reagents for use in the treatment and/or prophylaxis of a range of conditions associated with aberrant differentiation, such as in cancer or autoimmune disease, or in harnessing normal developmental programs such as in the development of an immune response.

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SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Nucleotide and amino acid sequences are referred to by a sequence identifier number (SEQ ID NO:). The SEQ ID NOs: correspond numerically to the sequence identifiers <400>1 (SEQ ID NO:1), <400>2 (SEQ ID NO:2), etc. A summary of sequence identifiers is provided in Table 1. A sequence listing is provided after the claims.

Genes and other genetic material (eg mRNA, constructs etc) are represented in italics and their proteinaceous expression products are represented in non-italicised form. Thus, the transcription factor Blimp is the expression product of *Blimp*. The term "Blimp" or "*Blimp*" is used to denote all homolog molecules in any animal or mammalian species including the human homolog. Accordingly, human *PRDM-1* and its product, PRDM-1 is encompassed in the terms *Blimp* or Blimp.

The present invention is predicated, in part, on the identification of Blimp as being involved in the differentiation of haemopoietic and embryonic cells. By screening for the presence of Blimp, or level of Blimp, a determination can be made as to the stage of terminal differentiation of a cell. The identification of the role of Blimp further enables substantially homogeneous populations of particular haemopoietic cells to be identified such as, but not limited to, ASC (plasma cells).

Specifically, a genetically modified cell or non-human organism comprising such cells is provided by the present invention. The cells produce Blimp translated from an mRNA modified to encode a reporter molecule. Preferably, the reporter molecule encoding sequence is inserted into an intron of the Blimp allele. Such cells are useful in *in vivo* or *in vitro* cellular model systems to identify and isolate, *inter alia*, ASC.

In one aspect, the present invention provides a genetically modified cell or non-human organism comprising such cells comprising modified genetic material which when expressed produces a polypeptide co-expressed with a reporter molecule and wherein the polypeptide is associated with terminal differentiation of a haemopoietic cell. Preferably, the genetic material gene is a *Blimp* allele or a part, fragment or functional form thereof. Furthermore, the identification of the reporter molecule in B-cell lineage cells indicates that such cells are committed to differentiate or have differentiated into ASC. Alternatively, reporter molecule activity in cells of a T cell lineage indicates that these cells are activated.

Genetically modified non-human organisms may be provided in the form of embryos for transplantation. Embryos are preferably maintained in a frozen state and may optionally be sold with instructions for use. Targeting constructs and genetically modified cells are also preferably maintained in a frozen state and may optionally be sold with instructions for use. All such cells are referred to herein as an *in vivo* or *in vitro* cellular model system.

The present invention provides a system for monitoring gene expression and differentiation fate in cells *in vivo* and *in vitro* at the single cell, tissue and organism level. Thus, reporter activity may be monitored in live cells and gene expression monitored in fixed tissues. Preferably, the reporter expression cassette encodes a fluorescent or other light emitting moiety. The availability of organisms and cells which report the expression of *Blimp-1* for example as a marker for terminal differentiation of a particular lineage or cell will be an extremely useful tool in a wide range of applications. In relation to cells of the B-cell lineage, this system finds broad application in the study, isolation and monitoring of ASC. As previously mentioned, ASC have not hitherto been available for study although these cells are crucial for an effective antibody response. Furthermore, aberrant differentiation in ASC causes multiple myeloma in man.

In a related embodiment, the present invention provides a method for phenotyping and/or monitoring a cell of the haematopoietic system comprising screening a genetically

modified cell or non-human animal comprising such cells comprising a modified *Blimp* gene encoding a Blimp protein which when expressed co-expresses Blimp or a part, fragment or functional form thereof and a reporter molecule, wherein detection of reporter activity is indicative of a cellular phenotype and/or commitment of a cell to terminally differentiate. Haematopoietic cells include without limitation B-cells, T-cells, dendritic cells, macrophages, natural killer cells, granulocytes, erythrocytes, eosinophils, megakaryocytes, bone marrow, splenic, dermal, or stromal cells or their derivatives.

In a further embodiment, cells which exhibit reporter activity or changes in reporter activity are isolated or selected from among cells which do not exhibit reporter activity. Isolation of reporter-active cells may be by any convenient method. For example, flow cytometry, laser scanning cytometry, chromatography and/or other equivalent procedures are conveniently employed. Flow cytometric procedures are particularly preferred. Additionally, further selection markers such as for example drug selection markers, may be used to isolate or select the modified cells of the present invention. Preferably, the cells are ASC identified or isolated from a population of cells comprising substantially cells of of a B-cell lineage.

The present invention also provides a method for testing the antigenicity of a vaccine or the ability of agents to enhance or suppress antibody production by ASC wherein reduced reporter activity is indicative of an agent which down regulates or inhibits an antibody response and reporter activity or enhanced reporter activity relative to controls is indicative of agents which are positive regulators of the antibody response. In accordance with this aspect, the method comprises:

(i) administering an agent or vaccine to a genetically modified cell or non-human animal comprising such cells wherein the cell or organism comprises a modified *Blimp-1* gene which encodes a Blimp polypeptide which when expressed produces a Blimp polypeptide co-expressed with a reporter molecule;

(ii) testing the cell or organism for the reporter molecule, the presence of which is indicative of cellular phenotype and the ability of said agent or vaccine to regulate antibody production by ASC.

The present invention also provides antagonists and agonists of *Blimp-1* expression or Blimp-1 activity. Pharmaceutical compositions are further contemplated comprising recombinant, synthetic or isolated forms of the present agonists and antagonists and one or more pharmaceutically acceptable carriers, diluents or excipients. Reference to *Blimp-1* expression or production of Blimp-1 protein includes in a single cell or within a population of cells.

TABLE 1

Summary of sequence identifiers

SEQUENCE ID NO.	DESCRIPTION
1	Nucleotide sequence encoding murine Blimp-1
2	Amino acid sequence encoding murine Blimp-1
3	Nucleotide sequence encoding human Blimp-1 (PRDM-1)
4	Amino acid sequence encoding human Blimp-1 (PRDM-1)
5	Genomic nucleotide sequence of murine Blimp-1
6	Genomic nucleotide sequence of human Blimp-1 (PRDM-1)

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a diagrammatic representation of the Blimp-1 locus and a targeting strategy. A) Structural domains of the Blimp-1 protein. The segment of the protein encoded by exons 7-8 are indicated. Acidic, N and C terminal acidic regions; PR, region of homology to the retinoblastoma interacting zinc finger protein RIZ; Pro, proline rich region; Zn, 5 Zinc fingers. B) Genomic locus of Blimp-1, indicating the 8 exons as boxes and introns as black lines. Coding regions are in grey, non-translated regions are white. Restriction enzymes used for Southern hybridisations are marked, along with the 5' and 3' probes. Targeted allele derived from the homologous recombination event and subsequent manipulations is indicated C) Southern hybridisation on targeted and control ES cell DNA, using 5' and 3' ends of the Blimp-1 locus, to show expected products of the targeting event (4.8kb 5' arm and 4.5kb 3' arm). Expression of Blimp-1 in blimp$^{gfp/+}$ LPS stimulated B cells cultured for 0-3 days *ex vivo* in IL 15 +/-IL21. Blimp-1 expression was detected using a monoclonal antibody against mouse Blimp-1, a goat polyclonal antibody against α-actin was used as a loading control. +/+, wild type C57B1/6 mice; +/T blimp$^{gfp/+}$ mice.

Figure 2 is a graphical representation showing the results of FACS analysis of Blimp^{gfp} expression in B-cells *in vivo*. A. Syndecan-1 and Blimp^{gfp} expression in lymph nodes, spleen and bone marrow in Blimp$^{gfp/+}$ mice (upper panel) and controls (lower panel). B. Expression of Blimp^{gfp} in B220 positive B cells.

Figure 3 is a graphical and photographic representation showing the results of ELIspot analysis of Blimp^{gfp} sorted cells. Gfp positive cells were sorted from bone marrow (BM) and spleen of an untreated Blimp$^{gfp/+}$ mouse and analysed in an Elispot assay. Isotype specific antibodies or anti kappa antibodies were used to coat the elispot plate and to detect secreted immunglobulins. A - Distribution of isotype specific immunglobulins in 200 gfp-positive sorted cells (one representative experiment of three). B - Detection of kappa chain in a single representative well of an ELIspot plate (sample: sorted bone marrow cells). left, input 200 gfp-positive cells; middle, input 100 000 gfp-negative cells; right, input 100 000 unsorted cells.

Figure 4 is a graphical representation of the results of FACS analysis showing induction of antibody secreting cells with LPS *in vivo*. Blimp$^{gfp/+}$ mice were i.v. injected with 2ug E. coli LPS. Spleens (A) and bone marrows (B) of these mice were analysed at indicated time points after LPS treatment. LPS induces the formation of ASC, increasing the frequency from about 0.5% to about 5% at day 3 in spleen and from about 0.05% to about 0.25% at day 4 in the bone marrow, respectively. upper panel, FACS scans for syndecan-1 and Blimp^{gfp}. middle panel, syndecan-1 and B220 in gfp-positive gated cells. lower panel, histograms for syndecan-1 and B220 expression in GFP-positive cells at indicated time points.

Figure 5A is a graphical representation of the kinetics of Blimp^{gfp} expression. Flow cytometry histograms of Blimp^{gfp} expression by stimulated B cells from Blimp$^{gfp/+}$ mice (red line) and wild type C 57B1/6 mice (blue me) are shown. Histogram gates show a percentage of Blimp^{gfp} positive populations. Highly purified small resting B cells were stimulated recombinant CD40L, IL-4 and IL-5 (top panels) or LPS (20ug/ml) (bottom panels). Cells were harvested different days of culture time and analysed on flow cytometry. LPS stimulated cells start to express Blimp at 2 days, while in response to CD40L and IL4/IL5 Blimp expression become evident 3 days.

Figure 5B is a graphical representation showing that Blimp^{gfp} positive cells secrete antibodies. Blimp$^{gfp/+}$ B cells were stimulated with LPS for four days. Cells were harvested and stained with Syndecan-1 (Synd-1) specific antibodies and GFP expressing (left panel, A-C) and non-expressing regions (left panel, D) were sorted directly to the Elispot plates coated with various isotype specific antibodies, using automated cell deposition unit. Sorted cells were processed according to the standart Elispot method. Right panels show number of Ig secreting cells in sorted regions. Most Blimp^{gfp} cells secrete Ig, while all Blimp^{gfp} negative cells do not secrete any of Ig isotypes tested.

Figure 5C is a graphical representation showing the different expression of Blimp^{gfp} in response to various stimuli. Highly purified small resting B cells were stimulated with i)

re combinant CD40L and IL-4; ii) CD40L, IL4 and IL-5; iii) LPS; iv) LPS and IL-4; v) LPS and anti-IgD monoclonal antibody. After four days of culture cells were harvested, stained with Synd-1 specific antibody and analysed on flow cytometry. Shown here are two parameter dot plots of flow cytometry analysis.

Figure 6 is a graphical representation showing the results of analyses of mice transplanted with activated B-cells. Purified resting splenic B-cells of Blimp$^{gfp/+}$ mice were activated for three days in the presence of 20ug/ml LPS. 3x10^6 cells (containing about 2x10^6 gfp positive cells, i.e. antibody secreting cells, A) were washed three times with LPS and transplanted into WT recipients by i.v. injection. After 7 days the recipient mice were analysed for the presence of donor ASC (B).

Figure 7 A is a tabulated summary of genotyping results of mice born from Blimp$^{gfp/+}$ x blimp$^{gfp/+}$ matings. **Figure 7 B** is a photographical representation of Representative PCR results of genotyping of mice weaned (left) or embryos at day E9.5 (right).

Figure 8 is a photographic and graphical representation of splenocytes of Blimp$^{gfp/gft}$ and Blimp$^{gfp/+}$ reconstituted mice were cultured in the presence of 20ug/ml LPS and analysed for the presence of GFP positive, i.e. antibody secreting cells, at day three (A). GFP positive cells of both cultures were than sorted (B, gate R1) and analysed in an ELIspot assay. While Blimp$^{gfp/+}$ cells yielded 60-70% antibody secreting cells (B, lower panel left), Blimp$^{gfp/gft}$ gave only 5-7% antibody secreting cells which produced only tiny ELIspot's (B, lower panel, right) compared to spots produced by heterozygous cells. Detection of IgM and kappa chain in single representative wells of an ELISPOT plate (input 200 gfp-positive cells).

Figure 9 is a graphical representation of the results of FACS analysis of bone marrow derived macrophages (BMM) and blood monocytes. Bone marrow cells were cultured for 7 days in the presence of 10ng/ml rMCSF, medium was changed and non-adherent cells were removed at day 3 and 5 of culture. Adherent cells (BMM) were analysed for Blimp^{gfp}

expression (left panel). Further, MacI/Gr1 double positive blood cells were analysed in FACS (right panel) (black line - wildtype, red line - Blimp$^{gfp/+}$).

Figure 10 is a graphical representation showing FACS analysis *in vitro* generated dendritic cells (DC's). Bone marrow cells were cultured for 8 days in the presence of 100ng/ml Flt3 ligand. Cells were than cultured for another 24 hours (left column) or were stimulated with CpG (1.5uM), GMCSF (50ng/ml), gIFN (20ng/ml) and IL4 (20ng/ml) (middle column) or with 1ug/ml LPS (right column). Blimp^{gfp} expression is shown in a histograms for plasmacytoid DC's and conventional DC's (solid line - wildtype, dotted line - *Blimp*$^{gfp/+}$).

Figure 11 is a graphical representation showing FACS analysis of T cells *in vivo* and *in vitro*. Thymic (left) and lymph node (middle) T cells, and *in vitro* activated CD4+/CD8+ purified lymph node cells (right) of *Blimp*$^{gfp/+}$ mice were analysed in FACS. *Blimp*^{gfp} expression levels of gated T cell populations are shown in histograms (lower panel; black line - wildtype, red line - *Blimp*$^{gfp/+}$).

Figure 12 is a graphical representation showing *Blimp*-1 expression in the NK lineage can be detected in the *Blimp*$^{gfp/+}$ reporter mice and induced by maturation stimuli. A) *in vivo* splenic NK cells are GFP+. B) Sorted NK cells from *Blimp*$^{gfp/+}$ spleens were cultured for 4 days in IL 15, followed by 2 days in the indicated cytokine. mfi, mean fluorescence index of *Blimp*^{gfp}. C) Expression of *Blimp*-1 in +/+ NK cells cultured for 7 days *ex vivo* in IL15 +/-IL21. *Blimp*-1 expression was detected using a monoclonal antibody against mouse Blimp-1, a goat polyclonal antibody against α-actin was used as a loading control.

Figure 13 is a representation showing the cDNA and predicted amino acid sequence of mouse Blimp-1/PRDM-1. The coding sequence is shown in upper case.

Figure 14 is a representation showing the amino acid sequence of mouse Blimp-1/PRDM-1 derived from the nucleotide sequence (upper case) in Figure 13.

Figure 15 is a representation showing the cDNA and predicted amino acid sequence of human Blimp-1/PRDM-1. The coding sequence is shown in upper case.

Figure 16 is a representation showing the amino acid sequence of human Blimp-1/PRDM-1 derived from the nucleotide sequence (upper case) in Figure 15.

Figure 17 is a representation showing the genomic nucleotide sequence of mouse *Blimp-1*. The genomic locus comprises 8 exons in bold upper case. ATG and stop codons are underlined.

Figure 18 is a representation showing the genomic nucleotide sequence of human *Blimp-1*. The genomic locus comprises 8 exons in upper case, bold. ATG and stop codons are underlined.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is predicated, in part, by the development of a method for identifying and isolating cells of the haematopoietic system or embryonic cells and/or monitoring the differentiation of haematopoietic or embryonic cells, the method comprising detecting or quantifying the presence of a polypeptide (via a reporter) whose presence is associated with terminal differentiation of the cells.

In a particularly preferred embodiment, the polypeptide is Blimp or a part, fragment or functional form thereof which is co-expressed with a reporter molecule.

Accordingly, one aspect of the present invention provides a genetically modified cell or non-human organism comprising such cells comprising genetic material encoding a polypeptide which when expressed produces the polypeptide co-expressed with a reporter molecule and which polypeptide is associated with a cellular phenotype including a commitment in the cell to terminally differentiate.

In a further aspect, the present invention provides a genetically modified cell or non-human organism comprising such cells comprising a modified *Blimp* gene encoding a Blimp polypeptide which when expressed produces Blimp or a part, fragment or functional form thereof co-expressed with a reporter molecule and wherein the presence of Blimp is associated with a cellular phenotype including a commitment in the cell to terminally differentiate.

In a further preferred aspect, the present invention provides a genetically modified cell or non-human organism comprising such cells comprising a modified *Blimp* gene encoding a Blimp mRNA transcript comprising a Blimp coding sequence or a part, fragment or functional form thereof and a reporter molecule encoding sequence, wherein the presence of Blimp is associated with a cellular phenotype including a commitment in the cell to terminally differentiate.

Preferably, the reporter molecule encoding sequence is inserted with an intron of the *Blimp* allele. In this way, the modified *Blimp* allele co-produces the reporter from a bicistronic RNA under the control of endogenous *Blimp* regulatory elements.

The terms "co-expression" and "co-production" are used herein in a broad sense to refer to the transcription of two or more nucleic acid regions (expressed as one or more RNAs) at the same time or at substantially the same time and their subsequent translation (produced as one or more polypeptides) at the same or substantially the same time. Preferably, one transcript is expressed which encodes both Blimp or a part, fragment or functional form thereof and a reporter molecule. In each case, the expression of the reporter is operatively linked to the expression of the molecule to be reported.

Reference to "cellular phenotype" herein encompasses the molecular or functional characteristics of a cell. For example, ASC cells express *Blimp-1* (a molecular marker) and are functionally distinguished from other B-cells by exhibiting, *inter alia*, a high rate of Ig secretion, the absence of MHC class II molecules and low levels of surface Ig. As used herein, the term is a reference to the full range of molecular or functional characteristics, or any particular molecules or functional characteristic in addition to the molecular characteristic of modulated levels of *Blimp-1* expression.

The genetically modified cell or non-human organism comprising such cells may comprise cells or genetic material from any organism such as, but not limited to, humans, non-human primates, livestock, companion or laboratory test organism, reptilian or amphibian species. Preferably the genetically modified organism is a mouse or other laboratory test animal such as a rat, guinea pig, pig, rabbit or sheep.

As used herein the singular forms "a", "an" and "the" include plural aspects unless the context clearly dictates otherwise. Thus, for example, reference to a "cell" includes a single cell, as well as two or more cells; reference to "a gene" includes a gene, as well as two or more gene; and so forth.

The modified gene of the present invention is a marker for terminal differentiation in cells of the haemopoietic system, such as B-cell lineage cells.

Reference to a "genetically modified cell" is a reference to any cell which has been engineered to comprise a sequence of nucleotides from a coding or non-coding region of the genome which is altered relative to its pre-modified form, and its progeny. In particular, the cell is genetically modified to co-express a genetic marker of terminal differentiation and a reporter molecule encoding sequence. Preferably, the cell is genetically modified to co-express Blimp or a part, fragment or functional part thereof and a reporter molecule. The reporter molecule may be any molecule capable of directly or indirectly providing an identifiable signal. A fluorescent or other light emitting reporter molecule is particularly preferred.

Conveniently, targeting constructs are initially used to generate the modified genetic sequences in the cell or organism. Targeting constructs generally but not exclusively modify a target sequence by homologous recombination. Alternatively, a modified genetic sequence may be introduced using artificial chromosomes. Targeting or other constructs are produced and introduced into target cells using methods well known in the art which are described in molecular biology laboratory manuals such as, for example, in Sambrook, *Molecular Cloning: A Laboratoy Manual*, 3rd Edition, CSHLP, CSH, NY, 2001; Ausubel (Ed) *Current Protocols in Molecular Biology*, 5th Edition, John Wiley & Sons, Inc, NY, 2002. Targeting constructs may be introduced into cells by any method such as electroporation, viral mediated transfer or microinjection. Selection markers are generally employed to initially identify cells which have successfully incorporated the targeting construct.

In one particular embodiment the present invention provides a nucleic acid construct suitable for use as a targeting construct said construct comprising all or a portion of an allele of *Blimp-1* and a reporter construct. The construct comprise genetic material which encodes a functionally active Blimp-1 polypeptide or a functionally inactive Blimp-1 polypeptide. In a particular embodiment, the construct encodes a partial Blimp-1

polypeptide which lacks a zinc finger domain comprising a DNA binding motif. In a particularly preferred embodiment, the construct is flanked by sites to facilitate recombinase mediated deletion and homologous recombination of the nucleic acid construct into a target genetic sequence. Alternatively, the construct may be introduced into a host cell where it replicates episomally.

Genetically modified organisms are generated using techniques well known in the art such as described in Hogan *et al., Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Sping Harbour Laboratory Press, CSH NY, 1986; Mansour *et al., Nature 336*:348-352, 1988; Pickert, *Transgenic Animal Technology: A Laboratory Handbook*, Academic Press, San Diago, CA, 1994. Stem cells including embryonic stem cells (ES cells) are introduced into the embryo of a recipient organism at the blastocyst stage of development. There they are capable of integration into the inner cell mass where they develop and contribute to the germ line of the recipient organism. ES cells are conveniently obtained from pre-implantation embryos maintained *in vitro* (Robertson *et al., Nature 322*:445-448, 1986). Once correct targeting has been verified, modified cells are injected into the blastocyst or morula or other suitable developmental stage, to generate a chimeric organism. Alternatively, modified cells are allowed to aggregate with dissociated embronic cells to form aggregation chimera. The chimeric organism is then implanted into a suitable female foster organism and the embryo allowed to develop to term. Chimeric progeny are bred to obtain offspring in which the genome of each cell contains the nucleotide sequences conferred by the targeting construct. Genetically modified organism may comprise a heterozygous modification or alternatively both alleles may be affected.

In accordance with the present invention it is surprisingly determined that Blimp-1 is essential for the production of antibody by ASC but not the commitment to differentiate down the ASC pathway. Accordingly, the identification of Blimp (eg via a reporter molecule co-expressed therewith) in B-cell lineage cells indicates that the cells are committed to differentiate or have differentiated into ASC.

Accordingly, another aspect of the present invention provides a genetically modified cell or non-human organism comprising such cells comprising genetic material encoding a polypeptide which when expressed produces the polypeptide co-expressed with a reporter molecule wherein detection of said reporter molecule is indicative of a cellular phenotype and/or commitment of a cell to terminally differentiate.

In a further aspect, the present invention provides a genetically modified cell or non-human organism comprising such cells comprising a modified *Blimp* gene encoding a Blimp polypeptide which when expressed produces Blimp or a part, fragment or functional form thereof co-expressed with a reporter molecule and wherein detection of said reporter molecule is indicative of a cellular phenotype and/or commitment of a cell to terminally differentiate.

In a further preferred aspect, the present invention provides a genetically modified cell or non-human organism comprising such cells comprising a modified *Blimp* gene encoding a Blimp mRNA transcript comprising a Blimp coding sequence or a part, fragment or functional form thereof and a reporter molecule encoding sequence, wherein detection of said reporter molecule is indicative of a cellular phenotype and/or commitment of a cell to terminally differentiate

Preferably, the reporter molecule encoding sequence is inserted with an intron of the *Blimp* allele. In this way, the modified *Blimp* allele co-produces the reporter from a bicistronic RNA under the control of endogenous *Blimp* regulatory elements.

Accordingly, another aspect of the present invention provides a genetically modified cell or non-human organism comprising such cells comprising genetic material encoding a polypeptide which when expressed produces the polypeptide co-expressed with a reporter molecule and wherein detection of said reporter molecule in cells of the haemopoietic system is indicative of a cellular phenotype and/or commitment of a cell to terminally differentiate.

In a further aspect, the present invention provides a genetically modified cell or non-human organism comprising such cells comprising a modified *Blimp* gene encoding a Blimp polypeptide which when expressed produces Blimp or a part, fragment or functional form thereof co-expressed with a reporter molecule and wherein detection of said reporter molecule in B-cells is indicative that cells having reporter molecule activity are committed to differentiation into ASC.

In a further preferred aspect, the present invention provides a genetically modified cell or non-human organism comprising such cells comprising a modified *Blimp* gene encoding a Blimp mRNA transcript comprising a Blimp coding sequence or a part, fragment or functional form thereof and a reporter molecule encoding sequence, wherein detection of said reporter molecule in T-cells is indicative that cells having reporter molecule activity are activated T-cells.

Preferably, the reporter molecule encoding sequence is inserted with an intron of the *Blimp* allele. In this way, the modified *Blimp* allele co-produces the reporter from a bicistronic RNA under the control of endogenous *Blimp* regulatory elements.

Reference herein to a *Blimp-1* gene or nucleic acid expression product thereof (RNA) includes homologs, parts, fragments, functional forms thereof including functional variants or derivatives which hybridize thereto under low stringency conditions or comprise significant sequence similarity to all or a functional part such as at least about 60% sequence similarity, after optimal alignment. Reference to a Blimp-1 polypeptide or protein is used in a broad sense to include all homologs, parts, fragments or functional forms thereof including functional variants or derivatives bearing at least about 60% amino acid sequence similarity after optimal alignment.

Functional parts of the instant molecules include portions of the full length molecule which are important for the particular functions thereof such as substrate binding, tertiary conformation or transcriptional activity. Transcription initiation sites are readily mapped and sites conferring promoter activity readily identified (see for example Tunyaplin *et al.*,

Nucleic Acid Research 28(24):4846-4855, 2000). Functional parts are important for regulating the expression and activity of the molecule. Functional variants or derivatives retain at least one of the functional activities important for regulating expression and activity of the parent molecules. With reference to *Blimp-1*, its expression is associated with terminal differentiation, induction of Ig secretion by ASC cells and activation of T-cells.

The modified *Blimp* gene may encode a functionally active Blimp polypeptide, a functionally inactive Blimp polypeptide and/or partial Blimp polypeptide such as a polypeptide or peptide, for example, lacking a zinc finger domain comprising a DNA binding motif. The terms "polypeptide" and "protein" are used interchangeably herein.

A "part" in peptide form may be as small as an epitope comprising less than 5 amino acids or as large as several hundred kilodaltons. The length of the polypeptide sequences compared for homology will generally be at least about 16 amino acids, usually at least about 20 residues, more usually at least about 24 residues, typically at least about 28 residues and preferably more than about 35 residues. A "part" of a nucleic acid molecule is defined a having a minimal size of at least about 10 nucleotides or preferably about 13 nucleotides or more preferably at least about 20 nucleotides and may have a minimal size of at least about 35 nucleotides. This definition includes all sizes in the range of 10-35 nucleotides as well as greater than 35 nucleotides including 50, 100, 300, 500, 600 nucleotides or nucleic acid molecules having any number of nucleotides within these values.

The present invention encompasses Blimp from any mammal or animal (including avian species) subject such as from humans, non-human primates, livestock, laboratory, companion or wild animals. Reference to "Blimp" includes Blimp or *Blimp* from any of the above species as well as structural or evolutionary equivalents or homologs thereof. for example, the present invention encompasses Blimp or a *Blimp* having an amino acid sequence which has substantially at least about 60% similarity to SEQ ID NO: 2 or 4 or at least about 60% identity to SEQ ID NO:1, 3, 5 or 6. Reference to at least about 60%

includes 60, 61, 62, 63, 64% and all following consecutive numbers in the series to 100%.

Function derivatives of molecules in nucleic acid form include nucleic acid molecules comprising a nucleotide sequence capable of hybridising to the molecule or its complementary form under low stringency conditions.

The terms "similarity" or identity as used herein includes exact identity between compared sequences at the nucleotide or amino acid level. Where there is non-identity at the nucleotide level, "similarity" includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. Where there is non-identity at the amino acid level, "similarity" includes amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. In a particularly preferred embodiment, nucleotide and amino acid sequence comparisons are made at the level of identity rather than similarity.

Terms used to describe sequence relationships between two or more polynucleotides or polypeptides include "reference sequence", "comparison window", "sequence similarity", "sequence identity", "percentage of sequence similarity", "percentage of sequence identity", "substantially similar" and "substantial identity". A "reference sequence" is at least 12 but frequently 15 to 18 and often at least 25 or above, such as 30 monomer units, inclusive of nucleotides and amino acid residues, in length. Because two polynucleotides may each comprise (1) a sequence (i.e. only a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window" refers to a conceptual segment of typically 12 contiguous residues that is compared to a reference sequence. The comparison window may comprise additions or deletions (i.e. gaps) of about 20% or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment

of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by computerised implementations of algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, WI, USA) or by inspection and the best alignment (i.e. resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as, for example, disclosed by Altschul *et al., Nucl. Acids Res. 25:* 3389, 1997. A detailed discussion of sequence analysis can be found in Unit 19.3 of Ausubel *et al., Current Protocols in Molecular Biology* John Wiley & Sons Inc, 1994-1998, Chapter 15).

The terms "sequence similarity" and "sequence identity" as used herein refer to the extent that sequences are identical or functionally or structurally similar on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity", for example, is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g. A, T, C, G, I) or the identical amino acid residue (e.g. Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity. For the purposes of the present invention, "sequence identity" will be understood to mean the "match percentage" calculated by the DNASIS computer program (Version 2.5 for windows; available from Hitachi Software engineering Co., Ltd., South San Francisco, California, USA) using standard defaults as used in the reference manual accompanying the software. Similar comments apply in relation to sequence similarity.

Furthermore, a *Blimp* homolog or derivative may be defined as being capable of hybridising to SEQ ID NO: 1, 3, 5 or 6 or to a complementary form thereof under low stringency conditions.

Reference herein to a low stringency includes and encompasses from at least about 0 to at least about 15% v/v formamide and from at least about 1 M to at least about 2 M salt for hybridization, and at least about 1 M to at least about 2 M salt for washing conditions. Generally, low stringency is at from about 25-30°C to about 42°C. The temperature may be altered and higher temperatures used to replace formamide and/or to give alternative stringency conditions. Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5 M to at least about 0.9 M salt for hybridization, and at least about 0.5 M to at least about 0.9 M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01 M to at least about 0.15 M salt for hybridization, and at least about 0.01 M to at least about 0.15 M salt for washing conditions. In general, washing is carried out $T_m = 69.3 + 0.41$ (G+C)% (Marmur *et al., J. Mol. Biol. 5:* 109, 1962). However, the T_m of a duplex DNA decreases by 1°C with every increase of 1% in the number of mismatch base pairs (Bonner *et al., Eur. J. Biochem. 46:* 83, 1974). Formamide is optional in these hybridization conditions. Accordingly, particularly preferred levels of stringency are defined as follows: low stringency is 6 x SSC buffer, 0.1% w/v SDS at 25-42°C; a moderate stringency is 2 x SSC buffer, 0.1% w/v SDS at a temperature in the range 20°C to 65°C; high stringency is 0.1 x SSC buffer, 0.1% w/v SDS at a temperature of at least 65°C.

Preferably the modified *Blimp* gene is modified using a nucleic acid construct comprising all or a portion of an allele of *Blimp* into which a nucleotide sequence encoding a reporter molecule is inserted.

The reporter molecule is conveniently encoded by a reporter expression cassette or reporter construct. The reporter construct can be brought under the control of the *Blimp-1* regulatory elements and faithfully report the *Blimp-1* expression pattern in cells, tissues or organisms.

By "reporter" is meant any molecule, protein or polypeptide which is typically encoded by a reporter gene and measured in a reporter assay. Reporters provide a detectable signal which permit an understanding of the activity of genetic sequences. They may report an activity directly or may indirectly monitor activity by monitoring the activity of down stream targets. A reporter protein should be distinguishable from other proteins and ideally, readily quantified. The reactivity between an epitope and an antibody determined thereby may readily be employed optionally together with second or further antibodies. Common reporter proteins include luciferase, chloramphenicol transferase (CAT), Beta-galactosidase (B-gal), or fluorescent proteins such as green fluorescent proteins (GFP). Reference herein to GFP is meant to encompass any fluorescent or light-emitting protein including those derived from jelly fish or other organisms and all homologues, derivatives, analogues including colour variants such as DSRed, HcRed, Clontech; or hrGFP, Stratagene). Preferably said reporter expression cassette encodes a fluorescent or other light emitting GFP. GFP reporters are readily detectable in live cells and are particularly useful and preferred in cell sorting applications.

Examples of fluorescent or light emitting markers may be selected from among those included, but are not limited to those, in the following Table 2.

TABLE 2

Probe	Ex. (nm)	Em. (nm)
Reactive and conjugated probes		
Hydroxycoumarin	325	386
Aminocoumarin	350	455
Methoxycoumarin	360	410
Cascade Blue	375; 400	423
Lucifer Yellow	425	528
NBD	466	539
R-Phycoerythrin (PE)	480; 565	578
PE-Cy5 conjugates	480; 565; 650	670
PE-Cy7 conjugates	480; 565; 743	767
APC-Cy7 conjugates	650; 755	767
Red 613	480; 565	613
Fluorescein	495	519

Probe	Ex^1 (nm)	Em^2 (nm)
FluorX	494	520
BODIPY-FL	503	512
TRITC	547	574
X-Rhodamine	570	576
Lissamine Rhodamine B	570	590
PerCP	490	675
Texas Red	589	615
Allophycocyanin (APC)	650	660
TruRed	490, 675	695
Alexa Fluor 350	346	445
Alexa Fluor 430	430	545
Alexa Fluor 488	494	517
Alexa Fluor 532	530	555
Alexa Fluor 546	556	573
Alexa Fluor 555	556	573
Alexa Fluor 568	578	603
Alexa Fluor 594	590	617
Alexa Fluor 633	621	639
Alexa Fluor 647	650	688
Alexa Fluor 660	663	690
Alexa Fluor 680	679	702
Alexa Fluor 700	696	719
Alexa Fluor 750	752	779
Cy2	489	506
Cy3	(512); 550	570; (615)
Cy3,5	581	596; (640)
Cy5	(625); 650	670
Cy5,5	675	694
Cy7	743	767
Nucleic acid probes		
Hoeschst 33342	343	483
DAPI	345	455
Hoechst 33258	345	478
SYTOX Blue	431	480
Chromomycin A3	445	575
Mithramycin	445	575
YOYO-1	491	509
SYTOX Green	504	523
SYTOX Orange	547	570
Ethidium Bormide	493	620
7-AAD	546	647
Acridine Orange	503	530/640
TOTO-1, TO-PRO-1	509	533
Thiazole Orange	510	530

Probe	Ex[1] (nm)	Em[2] (nm)
Propidium Iodide (PI)	536	617
TOTO-3, TO-PRO-3	642	661
LDS 751	543; *590*	712; *607*
Cell function probes		
Indo-1	361/330	490/405
Fluo-3	506	526
DCFH	505	535
DHR	505	534
SNARF	548/579	587/635
Fluorescent Proteins		
Y66F	360	508
Y66H	360	442
EBFP	380	440
Wild-type	396, 475	50, 503
GFPuv	385	508
ECFP	434	477
Y66W	436	485
S65A	471	504
S65C	479	507
S65L	484	510
S65T	488	511
EGFP	489	508
EYFP	514	527
DsRed	558	583
Other probes		
Monochlorobimane	380	461
Calcein	496	517

[1] Ex: Peak excitation wavelength (nm)

[2] Em: Peak emission wavelength (nm)

Any suitable method of analyzing fluorescence emission is encompassed by the present invention. In this regard, the invention contemplates techniques including but not restricted to 2-photon and 3-photon time resolved fluorescence spectroscopy as, for example, disclosed by Lakowicz *et al., Biophys. J. 72:* 567, 1997, fluorescence lifetime imaging as, for example, disclosed by Eriksson *et al., Biophys. J. 2:* 64, 1993, incorporated herein by reference) and fluorescence resonance energy transfer as, for example, disclosed by Youvan *et al., Biotechnology et Elia 3:* 1-18, 1997).

Exemplary fluorophores which may be used in accordance with the present invention include those discussed by Dower *et al.* (International Patent Publication No. WO 93/06121). Preferably, fluorescent dyes are employed. Any suitable fluorescent dye may be used for incorporation into the instant reporter molecule. For example, reference may be made to U.S. Patent Nos. 5,573,909 (Singer *et al.*) and 5,326,692 (Brinkley *et al.)* which describe a plethora of fluorescent dyes. Reference may also be made to fluorescent dyes described in U.S. Patent Nos. 5,227,487, 5,274,113, 5,405,975, 5,433,986, 5,442,045, 5,451,663, 5,453,517, 5,459,276, 5,515,864, 5,648,270 and 5,723,218.

A modern flow cytometer is able to perform these tasks up to 100,000 cells/particles s^{-1}. Through the use of an optical array of filters and dichroic mirrors, different wavelengths of fluorescent light can be separated and detected simultaneously. In addition, a number of lasers with different excitation wavelengths may be used. Hence, a variety of fluorophores can be used to target and examine, for example, intra- and extra-cellular properties of individual cells. The scattered light measurements can also classify an individual cells's size, shape, granularity and/or complexity and, hence, belonging to a particular population of interest (Shapiro, *Practical flow cytometry*, 3rd Ed., Brisbane, Wiley-Liss, 1995).

Suitable flow cytometers which may be used in the methods of the present invention include those which measure five to nine optical parameters (see Table 3) using a single excitation laser, commonly an argon ion air-cooled laser operating at 15 mW on its 488 nm spectral line. More advanced flow cytometers are capable of using multiple excitation lasers such as a HeNe laser (633 nm) or a HeCd laser (325 nm) in addition to the argon ion laser (488 or 514 nm). Optical parameters, corresponding to different optically detectable/quantifiable attributes, for a carrier, may be measured by a flow cytometer to provide a matrix of qualitative and/or quantitative information, providing a code (or addressability in a multi-dimensional space) for the carrier.

For example, Biggs *et al.* (*Cytometry 36:* 36-45, 1999) have constructed an 11-parameter flow cytometer using three excitation lasers and have demonstrated the use of nine distinguishable fluorophores in addition to forward and side scatter measurements for

purposes of immunophenotyping (i.e. classifying) cells. The maximum number of parameters commercially available currently is 17: forward scatter, side scatter and three excitation lasers each with five fluorescence detectors. Whether all of the parameters can be adequately used depends heavily on the extinction coefficients, quantum yields and amount of spectral overlap between all fluorophores (Malemed *et al.*, "*Flow cytometry and sorting*", 2nd Ed., New York, Wiley-Liss, 1990). However, it will be understood that the present invention is not restricted to any particular flow cytometer or any particular set of parameters. In this regard, the invention also contemplates use in place of a conventional flow cytometer, a microfabricated flow cytometer as, for example, disclosed by Fu *et al.*, *Nature Biotechnology 17:* 1109-1111, 1999.

TABLE 3

Exemplary optical parameters which may be measured by a flow cytometer.

Parameter	Acronym	Detection angle from incident laser beam	Wavelength (nm)
Forward scattered light	FS	2-5°	488*
Side scattered light	SS	90°	488*
"Green" fluorescence	FL1	90°	510-540†
"Yellow" fluorescence	FL2	90°	560-580†
"Red" fluorescence	FL3	90°	>650#

* using a 488 nm excitation laser

† width of bandpass filter

\# longpass filter

A flow cytometer with this capacity to sort is known as a "fluorescence-activated cell sorter" (FACS). Accordingly, the step of sorting in the present method of obtaining a population of detectably unique carriers may be effected by flow cytometric techniques such as by fluorescence activated cell sorting (FACS) although with respect to the present invention, FACS is more accurately "fluorescence activated carrier or solid support sorting" (see, for example, *"Methods in Cell Biology"* Vol. 33, Darzynkiewica, Z. and Crissman, H.A., eds., Academic Press)

In a further embodiment the present invention provides a method for phenotyping and/or monitoring a cell of the haematopoietic system comprising screening a genetically modified cell or non-human organism comprising such cells comprising a modified *Blimp* gene wherein expression or activity of said gene is indicative of a cellular phenotype and/or a commitment of said cell to terminally differentiate. Haemopoietic cells include but are not limited to B-cells, T-cells, dendritic cells, macrophages and natural killer cells, granulocytes, eosinophils, erythrocytes, megakaryocytes, bone marrow, stromal, splenic precursor cells and their derivatives.

Preferably the modified *Blimp* gene encodes a Blimp mRNA transcript comprising a Blimp coding sequence or a part, fragment or functional form thereof and a reporter molecule encoding sequence which when expressed produces Blimp or a part, fragment or functional form thereof co-expressed with a reporter molecule and wherein detection of the reporter molecule is indicative of cellular phenotpye and/or commitment of a cell to terminally differentiate.

In a further embodiment, cells which exhibit reporter activity or changes in reporter activity are isolated or selected from among cells which do not exhibit reporter activity. Isolation of reporter-active cells may be by flow cytometry, laser scanning cytometry, chromatography and/or other equivalent procedures. Additionally, further selection markers may be used to isolate or select the modified cells of the present invention. Flow cytometric isolation is particularly preferred.

Preferably the cells are ASC identified or isolated in a population of cells of a B-cell lineage.

Accordingly, the present invention provides a method for isolating a substantially purified population of ASC from a population of substantially B-cells said method comprising contacting a genetically modified cell or non-human organism comprising such cells wherein expression or activity of said gene is reported by a reporter construct and wherein detection of said reporter activity is indicative that cells with reporter molecule activity are ASC with an agent or composition capable of inducing differentiation to ASC, where necessary isolating B-cells from said organism and isolating ASC based on the activity of the reporter molecule.

Preferably the modified cell comprises a modified *Blimp* gene encoding a Blimp mRNA transcript comprising a Blimp coding sequence or a part, fragment or functional form thereof and a reporter molecule encoding sequence which when expressed produces Blimp or a part, fragment or functional form thereof co-expressed with a reporter molecule and wherein reporter activity is indicative that cells with reporter molecule activity are ASC.

Preferably, screening of cells is achieved by flow cytometric analysis of a fluroescent reporter molecule.

B-cells are conveniently isolated from an organism or sample for example by density gradient centrifugation, flow cytometry or using magnetic beads. Any agent or composition which selectively, clonally or polyclonally of otherwise effectively activates B-cells and induces their differentiation to ASC is encompassed. An example of a polyclonal activator is LPS.

In one embodiment the reporter is a GFP and said ASC are isolated by flow cytometry.

Substantially purified means that the ASC comprise at least about 60 to 95%, preferably at least about 97%, more preferably at least about 99% of the cells, such as at least about 60, 61, 62, 63, 64 and following subsequent numbers in the series to 100%. Alternatively, enrichment of approximately 100,000 fold over unsorted cells is contemplated.

The present invention also provides a method for testing the antigenicity of a vaccine or the ability of agents to enhance or suppress antibody production by ASC wherein reduced reporter activity is indicative of an agent which down regulates or inhibits an antibody response and reporter activity or enhanced reporter activity relative to controls is indicative of agents which are positive regulators of the antibody response. In accordance with this aspect, the method comprises:

(i) administering an agent or vaccine to a genetically modified cell or non-human animal comprising such cells wherein the cell or organism comprises a modified *Blimp-1* gene which encodes a Blimp polypeptide which when expressed produces Blimp or a part or fragment or functional form thereof co-expressed with a reporter molecule;

(ii) testing the cell or organism for the reporter molecule the presence of which is indicative of cellular phenotype and the ability of said agent or vaccine to regulate antibody production by ASC.

Preferrably the modified cell comprises a modified *Blimp* gene encoding a Blimp mRNA transcript comprising a Blimp coding sequence or a part, fragment or functional form thereof and a reporter molecule encoding sequence which when expressed produces Blimp or a part, fragment or functional form thereof co-expressed with a reporter molecule and wherein reporter activity is indicative that cells with reporter molecule activity are ASC.

The present invention also directed to antagonists and agonists of terminal differentiation of cells such as, but not limited to ASC including antagonists and agonists of *Blimp-1* expression or Blimp-1 activity, identified by the herein described method, for use in modulating cellular differentiation. The molecules to which the instant modulators, agonists or antagonists are directed are collectively referred to herein as "targets" or "target molecules".

"Modulation" of a molecule or differentiation status includes completely or partially inhibiting or reducing or down regulating all or part of its functional activity or differentiation and enhancing or up regulating all or part its functional activity or differentiation. Where the molecule is a genetic sequence its functional activity may be modulated by, for example, modulating its binding capabilities or transcriptional or translational activity, or its half-life. Where the molecule is an encoded polypeptide, its functional activity may be modulated by, for example, modulating its binding capabilities, its half-life, location in a cell or membrane or its enzymatic capability. Modulators are agonists or antagonists which achieve modulation. Enhanced differentiation can also be indicative of reduced cell division.

An example of an antagonist or agonist is a protein, polypeptide or peptide. These terms may be used interchangeably. These terms refer to a polymer of amino acids and its equivalent and does not refer to a specific length of the product, thus, polypeptides,

peptides, oligopeptides and proteins are included within the one definition of a polypeptide. These terms also do not exclude modifications of the polypeptide, for example, glycosylations, aceylations, phosphorylations and the like. Included within the definition are, for example, polypeptides containing one or more analogs of an amino acid including, for example, unnatural amino acids such as those given in Table 4 or polypeptides with substituted linkages. Such polypeptides may need to be able to enter the cell. Polypeptides carrying chemical analogs of the amino acids may be more resistant to protease mediated digestion. One example of an antagonist or agonist is a chemical analog of Blimp. Antagonists and agonists may affect the molecules with which Blimp interacts, such as, for example *c-myc* expression is repressed by Blimp-1.

Genetic molecules are also developed into agonist and antagonist modulators. The terms "genetic molecule" "nucleic acids", "nucleotide" and "polynucleotide" include RNA, cDNA, genomic DNA, synthetic forms and mixed polymers, both sense and antisense strands, and may be chemically or biochemically modified or may contain non-natural or derivatized nucleotide bases, as will be readily appreciated by those skilled in the art. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analog (such as the morpholine ring), internucleotide modifications such as uncharged linkages (e.g. methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.), charged linkages (e.g. phosphorothioates, phosphorodithioates, etc.), pendent moieties (e.g. polypeptides), intercalators (e.g. acridine, psoralen, etc.), chelators, alkylators and modified linkages (e.g. α-anomeric nucleic acids, etc.). Also included are synthetic molecules that mimic polynucleotides in their ability to bind to a designated sequence via hydrogen binding and other chemical interactions. Such molecules are known in the art and include, for example, those in which peptide linkages substitute for phosphate linkages in the backbone of the molecule. modifications of antisense molecules are well known and are summarised in Kurrek *Eur. J. Biochem. 270*:1628-1644,2003.

Antisense polynucleotide sequences, for example, are useful in silencing transcripts. Furthermore, polynucleotide vectors containing all or a part of an Blimp gene locus may be

placed under the control of a promoter in an antisense orientation and introduced into a cell. Expression of such an antisense construct within a cell will interfere with target transcription and/or translation. Furthermore, co-suppression and mechanisms to induce RNAi or siRNA may also be employed. Alternatively, antisense or sense molecules may be directly administered. In this latter embodiment, the antisense or sense molecules may be formulated in a composition and then administered by any number of means to target cells.

A variation on antisense and sense molecules involves the use of morpholinos, which are oligonucleotides composed of morpholine nucleotide derivatives and phosphorodiamidate linkages (for example, Summerton and Weller, Antisense and Nucleic Acid Drug Development 7: 187-195, 1997). Such compounds are injected into embryos and the effect of interference with mRNA is observed.

In one embodiment, the present invention employs compounds such as oligonucleotides and similar species for use in modulating the function or effect of nucleic acid molecules encoding Blimp or other suitable markers of terminal differentiation in a cell i.e. the oligonucleotides induce transcriptional or post-transcriptional gene silencing. This is accomplished by providing oligonucleotides which specifically hybridize with one or more nucleic acid molecules encoding the endogenous ligands. The oligonucleotides may be provided directly to a cell or generated within the cell. As used herein, the terms "target nucleic acid" and "nucleic acid molecule encoding an inhibitor" have been used for convenience to encompass DNA encoding the inhibitor, RNA (including pre-mRNA and mRNA or portions thereof) transcribed from such DNA, and also cDNA derived from such RNA. The hybridization of a compound of the subject invention with its target nucleic acid is generally referred to as "antisense". Consequently, the preferred mechanism believed to be included in the practice of some preferred embodiments of the invention is referred to herein as "antisense inhibition." Such antisense inhibition is typically based upon hydrogen bonding-based hybridization of oligonucleotide strands or segments such that at least one strand or segment is cleaved, degraded, or otherwise rendered inoperable. In this regard, it

is presently preferred to target specific nucleic acid molecules and their functions for such antisense inhibition.

The functions of DNA to be interfered with can include replication and transcription. Replication and transcription, for example, can be from an endogenous cellular template, a vector, a plasmid construct or otherwise. The functions of RNA to be interfered with can include functions such as translocation of the RNA to a site of protein translation, translocation of the RNA to sites within the cell which are distant from the site of RNA synthesis, translation of protein from the RNA, splicing of the RNA to yield one or more RNA species, and catalytic activity or complex formation involving the RNA which may be engaged in or facilitated by the RNA. In one example, the result of such interference with target nucleic acid function is reduced levels of Blimp or other suitable marker of terminal differentiation. In the context of the present invention, "modulation" and "modulation of expression" mean either an increase (stimulation) or a decrease (inhibition) in the amount or levels of a nucleic acid molecule encoding the gene, e.g., DNA or RNA. Inhibition is often the preferred form of modulation of expression and mRNA is often a preferred target nucleic acid.

An antisense compound is specifically hybridizable when binding of the compound to the target nucleic acid interferes with the normal function of the target nucleic acid to cause a loss of activity, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target nucleic acid sequences under conditions in which specific binding is desired, i.e. under physiological conditions in the case of in vivo assays or therapeutic treatment, and under conditions in which assays are performed in the case of in vitro assays.

"Complementary" as used herein, refers to the capacity for precise pairing between two nucleobases of an oligomeric compound. For example, if a nucleobase at a certain position of an oligonucleotide (an oligomeric compound), is capable of hydrogen bonding with a nucleobase at a certain position of a target nucleic acid, said target nucleic acid being a DNA, RNA, or oligonucleotide molecule, then the position of hydrogen bonding between

the oligonucleotide and the target nucleic acid is considered to be a complementary position. The oligonucleotide and the further DNA, RNA, or oligonucleotide molecule are complementary to each other when a sufficient number of complementary positions in each molecule are occupied by nucleobases which can hydrogen bond with each other. Thus, "specifically hybridizable" and "complementary" are terms which are used to indicate a sufficient degree of precise pairing or complementarity over a sufficient number of nucleobases such that stable and specific binding occurs between the oligonucleotide and a target nucleic acid.

According to the present invention, compounds include antisense oligomeric compounds, antisense oligonucleotides, ribozymes, external guide sequence (EGS) oligonucleotides, alternate splicers, primers, probes, and other oligomeric compounds which hybridize to at least a portion of the target nucleic acid. As such, these compounds may be introduced in the form of single-stranded, double-stranded, circular or hairpin oligomeric compounds and may contain structural elements such as internal or terminal bulges or loops. Once introduced to a system, the compounds of the invention may elicit the action of one or more enzymes or structural proteins to effect modification of the target nucleic acid. One non-limiting example of such an enzyme is RNAse H, a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. It is known in the art that single-stranded antisense compounds which are "DNA-like" elicit RNAse H. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of oligonucleotide-mediated inhibition of gene expression. Similar roles have been postulated for other ribonucleases such as those in the RNase III and ribonuclease L family of enzymes.

While the preferred form of antisense compound is a single-stranded antisense oligonucleotide, in many species the introduction of double-stranded structures, such as double-stranded RNA (dsRNA) molecules, has been shown to induce potent and specific antisense-mediated reduction of the function of a gene or its associated gene products. This phenomenon occurs in both plants and animals.

In the context of the subject invention, the term "oligomeric compound" refers to a polymer or oligomer comprising a plurality of monomeric units. In the context of this invention, the term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimetics, chimeras, analogs and homologs thereof. This term includes oligonucleotides composed of naturally occurring nucleobases, sugars and covalent internucleoside (backbone) linkages as well as oligonucleotides having non-naturally occurring portions which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for a target nucleic acid and increased stability in the presence of nucleases.

While oligonucleotides are a preferred form of the compounds of this invention, the present invention contemplates other families of compounds as well, including but not limited to oligonucleotides, analogs and mimetics such as those herein described.

The open reading frame (ORF) or "coding region" which is known in the art to refer to the region between the translation initiation codon and the translation termination codon, is a region which may be effectively targeted. Within the context of the present invention, one region is the intragenic region encompassing the translation initiation or termination codon of the open reading frame (ORF) of a gene.

Other target regions include the 5' untranslated region (5'UTR), known in the art to refer to the portion of an mRNA in the 5' direction from the translation initiation codon, and thus including nucleotides between the 5' cap site and the translation initiation codon of an mRNA (or corresponding nucleotides on the gene), and the 3' untranslated region (3'UTR), known in the art to refer to the portion of an mRNA in the 3' direction from the translation termination codon, and thus including nucleotides between the translation termination codon and 3' end of an mRNA (or corresponding nucleotides on the gene). The 5' cap site of an mRNA comprises an N7-methylated guanosine residue joined to the 5'-most residue of the mRNA via a 5'-5' triphosphate linkage. The 5' cap region of an mRNA is considered to include the 5' cap structure itself as well as the first 50 nucleotides

adjacent to the cap site. It is also preferred to target the 5' cap region.

As is known in the art, a nucleoside is a base-sugar combination. The base portion of the nucleoside is normally a heterocyclic base. The two most common classes of such heterocyclic bases are the purines and the pyrimidines. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to either the 2', 3' or 5' hydroxyl moiety of the sugar. In forming oligonucleotides, the phosphate groups covalently link adjacent nucleosides to one another to form a linear polymeric compound. In turn, the respective ends of this linear polymeric compound can be further joined to form a circular compound, however, linear compounds are generally preferred. In addition, linear compounds may have internal nucleobase complementarity and may, therefore, fold in a manner as to produce a fully or partially double-stranded compound. Within oligonucleotides, the phosphate groups are commonly referred to as forming the internucleoside backbone of the oligonucleotide. The normal linkage or backbone of RNA and DNA is a 3' to 5' phosphodiester linkage.

Specific examples of preferred antisense compounds useful in this invention include oligonucleotides containing modified backbones or non-natural internucleoside linkages. As defined in this specification, oligonucleotides having modified backbones include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified oligonucleotides that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides.

Preferred modified oligonucleotide backbones containing a phosphorus atom therein include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates, 5'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates,

thionoalkylphosphotriesters, selenophosphates and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein one or more internucleotide linkages is a 3' to 3', 5' to 5' or 2' to 2' linkage. Preferred oligonucleotides having inverted polarity comprise a single 3' to 3' linkage at the 3'-most internucleotide linkage i.e. a single inverted nucleoside residue which may be abasic (the nucleobase is missing or has a hydroxyl group in place thereof). Various salts, mixed salts and free acid forms are also included.

The isolated or recombinant agonists and antagonists of the instant invention are used directly or they may be further modified by methods well known in the art in order to improve their effectiveness as pharmaceutical or other reagents. Important considerations for an active compound include formulations and methods of delivery.

An agonist or antagonist includes molecules determined by all or part of the target in genetic or proteinaceous form, such as antibodies, mimetics or antisense molecules.

Antibodies including anti-idiotypic antibodies, chaemeric antibodies and humanised antibodies are useful in this regard and their generation is now routine to those of skill in the art. Peptide or non-peptide mimetics can be developed as agonists of the targets by identifying those residues of the target molecule which are important for function. Modelling can be used to design molecules which interact with the target molecule and which have improved pharmacological properties. All such molecules will need to be modified to permit entry into a cell.

Rational drug design permits the production of structural analogs of biologically active polypeptides of interest or of small molecules with which they interact (e.g. agonists, antagonists, inhibitors or enhancers) in order to fashion drugs which are, for example, more active or stable forms of the polypeptide, or which, e.g. enhance or interfere with the function of a polypeptide *in vivo*. See, e.g. Hodgson (*Bio/Technology 9:* 19-21, 1991). In one approach, one first determines the three-dimensional structure of a protein of interest by x-ray crystallography, by computer modeling or most typically, by a combination of

approaches. Useful information regarding the structure of a polypeptide may also be gained by modeling based on the structure of homologous proteins. An example of rational drug design is the development of HIV protease inhibitors (Erickson *et al.*, *Science 249:* 527-533, 1990). In addition, target molecules may be analyzed by an alanine scan (Wells, *Methods Enzymol. 202:* 2699-2705, 1991). In this technique, an amino acid residue is replaced by Ala and its effect on the peptide's activity is determined. Each of the amino acid residues of the peptide is analyzed in this manner to determine the important regions of the peptide.

It is also possible to isolate a target-specific antibody, selected by a functional assay and then to solve its crystal structure. In principle, this approach yields a pharmacore upon which subsequent drug design can be based. It is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-ids would be expected to be an analog of the original receptor. The anti-id could then be used to identify and isolate peptides from banks of chemically or biologically produced banks of peptides. Selected peptides would then act as the pharmacore.

Analogs contemplated herein include but are not limited to modification to side chains, incorporating of unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecule or their analogs.

Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with $NaBH_4$; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with $NaBH_4$.

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitization, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acid, contemplated herein is shown in Table 4.

TABLE 4

Codes for non-conventional amino acids

Non-conventional amino acid	Code	Non-conventional amino acid	Code
α-aminobutyric acid	Abu	L-N-methylalanine	Nmala
α-amino-α-methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
aminocyclopropane-carboxylate	Cpro	L-N-methylasparagine	Nmasn
		L-N-methylaspartic acid	Nmasp
aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl-carboxylate	Norb	L-N-methylglutamine	Nmgln
		L-N-methylglutamic acid	Nmglu
cyclohexylalanine	Chexa	L-Nmethylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisolleucine	Nmile
D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
D-lysine	Dlys	L-N-methylthreonine	Nmthr
D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg
D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug

D-threonine	Dthr	L-norleucine	Nle
D-tryptophan	Dtrp	L-norvaline	Nva
D-tyrosine	Dtyr	α-methyl-aminoisobutyrate	Maib
D-valine	Dval	α-methyl-γ-aminobutyrate	Mgabu
D-α-methylalanine	Dmala	α-methylcyclohexylalanine	Mchexa
D-α-methylarginine	Dmarg	α-methylcylcopentylalanine	Mcpen
D-α-methylasparagine	Dmasn	α-methyl-α-napthylalanine	Manap
D-α-methylaspartate	Dmasp	α-methylpenicillamine	Mpen
D-α-methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
D-α-methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
D-α-methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
D-α-methylisoleucine	Dmile	N-amino-α-methylbutyrate	Nmaabu
D-α-methylleucine	Dmleu	α-napthylalanine	Anap
D-α-methyllysine	Dmlys	N-benzylglycine	Nphe
D-α-methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
D-α-methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
D-α-methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
D-α-methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
D-α-methylserine	Dmser	N-cyclobutylglycine	Ncbut
D-α-methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
D-α-methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
D-α-methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
D-α-methylvaline	Dmval	N-cylcododecylglycine	Ncdod
D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr

	D-N-methylhistidine	Dnmhis	N-(hydroxyethyl))glycine	Nser
	D-N-methylisoleucine	Dnmile	N-(imidazolylethyl))glycine	Nhis
	D-N-methylleucine	Dnmleu	N-(3-indolylyethyl)glycine	Nhtrp
	D-N-methyllysine	Dnmlys	N-methyl-γ-aminobutyrate	Nmgabu
5	N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
	D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
	N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
	N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
	N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
10	N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
	D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
	D-N-methyltyrosine	Dnmtyr	N-methyla-napthylalanine	Nmanap
	D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
	γ-aminobutyric acid	Gabu	N-(*p*-hydroxyphenyl)glycine	Nhtyr
15	L-*t*-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
	L-ethylglycine	Etg	penicillamine	Pen
	L-homophenylalanine	Hphe	L-α-methylalanine	Mala
	L-α-methylarginine	Marg	L-α-methylasparagine	Masn
	L-α-methylaspartate	Masp	L-α-methyl-*t*-butylglycine	Mtbug
20	L-α-methylcysteine	Mcys	L-methylethylglycine	Metg
	L-α-methylglutamine	Mgln	L-α-methylglutamate	Mglu
	L-α-methylhistidine	Mhis	L-α-methylhomophenylalanine	Mhphe
	L-α-methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
	L-α-methylleucine	Mleu	L-α-methyllysine	Mlys
25	L-α-methylmethionine	Mmet	L-α-methylnorleucine	Mnle
	L-α-methylnorvaline	Mnva	L-α-methylornithine	Morn
	L-α-methylphenylalanine	Mphe	L-α-methylproline	Mpro
	L-α-methylserine	Mser	L-α-methylthreonine	Mthr
	L-α-methyltryptophan	Mtrp	L-α-methyltyrosine	Mtyr
30	L-α-methylvaline	Mval	L-N-methylhomophenylalanine	Nmhphe

N-(N-(2,2-diphenylethyl) carbamylmethyl)glycine	Nnbhm	N-(N-(3,3-diphenylpropyl) carbamylmethyl)glycine	Nnbhc
1-carboxy-1-(2,2-diphenylethylamino)cyclopropane	Nmbc		

Crosslinkers can be used, for example, to stabilize 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with n=1 to n=6, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of C_α and N $_\alpha$-methylamino acids and the introduction of double bonds between C_α and C_β atoms of amino acids.

Natural product, combinatorial or phage display technologies are all available for screening for modulators. A huge choice of high through put screening methods are available which may be adapted to employ the cells of the present invention.

Two-hybrid screening is also useful in identifying other members of the genetic network acting with of Blimp-1. Target interactions and screens for modulators can be carried out using the yeast two-hybrid system, which takes advantage of transcriptional factors that are composed of two physically separable, functional domains. The most commonly used is the yeast GAL4 transcriptional activator consisting of a DNA binding domain and a transcriptional activation domain. Two different cloning vectors are used to generate separate fusions of the GAL4 domains to genes encoding potential binding proteins. The fusion proteins are co-expressed, targeted to the nucleus and if interactions occur, activation of a reporter gene *(e.g. lacZ)* produces a detectable phenotype. In the present case, for example, S. *cerevisiae is* co-transformed with a library or vector expressing a cDNA GAL4 activation domain fusion and a vector expressing a Myb pathway component fused to GAL4. If *lacZ is* used as the reporter gene, co-expression of the fusion proteins

will produce a blue colour. Small molecules or other candidate compounds which interact with a target will result in loss of colour of the cells. Reference may be made to the yeast two-hybrid systems as disclosed by Munder *et al.*, (*Appl. Microbiol. Biotechnol. 52(3):* 311-320, 1999) and Young *et al.*, *Nat. Biotechnol. 16(10):* 946-950, 1998). Molecules thus identified by this system are then re-tested in the genetically modified organisms or genetically modified cells of the present invention.

Pharmaceutical compositions for therapy are further contemplated comprising recombinant, synthetic or isolated forms of the present agonists and antagonists and one or more pharmaceutically acceptable carriers, diluents or excipients. The treatment of cancer or the modulation of an immune response are particularly contemplated.

The term therapy should be taken as a reference to treatment or prophylaxis of a condition or disease. The term "treating" and "ameliorating" are used interchangeably.

The terms "composition" or "agent" or "medicament" refer to a chemical compound that induces a desired pharmacological and/or physiological effect. The term also encompass pharmaceutically acceptable and pharmacologically active ingredients of those compounds specifically mentioned herein including but not limited to salts, esters, amides, prodrugs, active metabolites, analogs and the like. When the above term is used, then it is to be understood that this includes the active agent *per se* as well as pharmaceutically acceptable, pharmacologically active salts, esters, amides, prodrugs, metabolites, analogs, etc. The term "compound" is not to be construed narrowly but extends to peptides, polypeptides and proteins as well as genetic molecules such as RNA, DNA and mimetics and chemical analogs thereof.

The phrases "ameliorating a disease or condition" or "treatment" or "therapeutic" are used in the broadest context and include any measurable or statistically significant improvement in a disease or condition or one or more symptoms or frequency of symptoms of a disease or condition as well as complete recovery from the disease or elimination of a condition, its symptoms or its underlying cause. The present invention is applicable to a large range

of diseases or conditions and the skilled addressee must determine the precise parameters of the assessment of phenotypes on a case by case basis. Conditions may be associated with one or more diseased or they may not be so linked. The amelioration of a condition encompasses any desired physiological or psychological change.

An effective amount of the instant compositions is established best by those skilled in the art. The term "effective amount" of a compound as used herein mean a sufficient amount of the agent to provide the desired therapeutic or physiological effect. Undesirable effects, e.g. side effects, are sometimes manifested along with the desired therapeutic effect; hence, a practitioner balances the potential benefits against the potential risks in determining what is an appropriate "effective amount". The exact amount required will vary from subject to subject, depending on the species, age and general condition of the subject, mode of administration and the like. Thus, it may not be possible to specify an exact "effective amount". However, an appropriate "effective amount" in any individual case may be determined by one of ordinary skill in the art using only routine experimentation.

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The polypeptides, nucleic acids, antibodies, peptides, chemical analogs, agonists, antagonists or mimetics of the present invention can be formulated in pharmaceutic compositions which are prepared according to conventional pharmaceutical compounding techniques. See, for example, Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing, Company, Easton, PA, U.S.A.). The composition may contain the active agent or pharmaceutically acceptable salts of the active agent. These compositions may

comprise, in addition to one of the active substances, a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g. intravenous, oral, intrathecal, epineural or parenteral.

For oral administration, the compounds can be formulated into solid or liquid preparations such as capsules, pills, tablets, lozenges, powders, suspensions or emulsions. In preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, suspending agents, and the like in the case of oral liquid preparations (such as, for example, suspensions, elixirs and solutions); or carriers such as starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations (such as, for example, powders, capsules and tablets). Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be sugar-coated or enteric-coated by standard techniques. The active agent can be encapsulated to make it stable to passage through the gastrointestinal tract while at the same time allowing for passage across the blood brain barrier. See for example, International Patent Publication No. WO 96/11698.

For parenteral administration, the compound may dissolved in a pharmaceutical carrier and administered as either a solution or a suspension. Illustrative of suitable carriers are water, saline, dextrose solutions, fructose solutions, ethanol, or oils of animal, vegetative or synthetic origin. The carrier may also contain other ingredients, for example, preservatives, suspending agents, solubilizing agents, buffers and the like. When the compounds are being administered intrathecally, they may also be dissolved in cerebrospinal fluid.

The active agent is preferably administered in a therapeutically effective amount. The actual amount administered and the rate and time-course of administration will depend on the nature and severity of the condition being treated. Prescription of treatment, e.g.

decisions on dosage, timing, etc. is within the responsibility of general practitioners or specialists and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of techniques and protocols can be found in Remington's Pharmaceutical Sciences, *(supra)*.

Alternatively, targeting therapies may be used to deliver the active agent more specifically to certain types of cell, by the use of targeting systems such as antibodies or cell specific ligands. Targeting may be desirable for a variety of reasons, e.g. if the agent is unacceptably toxic or if it would otherwise require too high a dosage or if it would not otherwise be able to enter the target cells.

Instead of administering these agents directly, they could be produced in the target cell, e.g. in a viral vector such as described above or in a cell based delivery system such as described in U.S. Patent No. 5,550,050 and International Patent Publication Nos. WO 92/19195, WO 94/25503, WO 95/01203, WO 95/05452, WO 96/02286, WO 96/02646, WO 96/40871, WO 96/40959 and WO 97/12635. The vector could be targeted to the target cells or expression of expression products could be limited to specific cells, stages of development or cell cycle stages. The cell based delivery system is designed to be implanted in a patient's body at the desired target site and contains a coding sequence for the target agent. Alternatively, the agent could be administered in a precursor form for conversion to the active form by an activating agent produced in, or targeted to, the cells to be treated. See, for example, European Patent Application No. 0 425 731A and International Patent Publication No. WO 90/07936.

The present invention is further described by the following non-limiting Examples.

EXAMPLE 1

Generation of a Blimp-1 mutant allele (*Blimp*^{gfp})

A *Blimp-1* targeting construct was produced in which, inserted into the intron 3' to exon 6, is an *eGFP* expression cassette consisting of a splice acceptor, stop codons in all three reading frames, an internal ribosome entry site (IRES), the cDNA encoding *eGFP*, and the SV40 polyadenylation signal to terminate transcription. Also inserted into the intron is a *PGK-Neo*^{r} gene to allow for the selection of embryonic stem (ES) cells with an integrated targeting vector. The *eGFP* and *Neo*^{r} cassettes are flanked by *Frt* sites to allow flp recombinase-mediated deletion of the inserted DNA. C57BL/6 ES cells were electroporated with the *Blimp-1* targeting construct, resistant clones selected by G418 resistance and screened by Southern hybridisation to 5' and 3' genomic DNA probes (Figure 1C). Four correctly targeted clones carrying the *Blimp*^{gfp} allele (Figure 1C) were identified from 300 screened colonies. These were injected into BALB/c blastocysts to obtain chimeric founders. These chimeras have been bred, and germ-line transmission has been achieved with one clone (4F3).

EXAMPLE 2

A GFP reporter that allows the description of the expression pattern of *Blimp-1*

Blimp-1 was initially reported to be expressed solely in B-lymphocytes that have been induced to undergo ASC differentiation (Turner *et al., (supra)*). However, subsequent studies have revealed a broader expression pattern of Blimp-1 during embryogenesis (Chang *et al., Mech Dev 117:305*, 2002) and in myeloid cells (Chang *et al., (supra)*, 2000). The *Blimp*^{gfp} allele permits a fuller definition of the expression pattern of *Blimp-1*, both within the haematopoietic lineage and more broadly in the organism. The targeting strategy outlined above results in a *Blimp*^{gfp} allele that expresses GFP from a bicistronic mRNA under the control of the endogenous Blimp-1 regulatory elements and is thus predicted to recapitulate the full *Blimp-1* expression pattern. In addition this strategy interrupts the *Blimp-1* mRNA transcript to produce a truncated version of the Blimp-1 protein (exons 1-6) that lacks the Zinc finger domains containing the DNA binding motif. In agreement with

this, Western blotting of *Blimp*^{gfp}/+ B cells induced to differentiate with LPS *in vitro* demonstrated both the wild type and truncated Blimp-1 protein bands (Figure 1D). By monitoring *GFP* expression in live cells and Blimp-1 protein in fixed tissue, the gene activity and differentiation fate of B-lymphocytes *in vivo* and *in vitro* at the single cell level can be monitored.

EXAMPLE 3

In vivo expression of *Blimp*-1 in ASC

Examination of lymphoid tissues in *Blimp*^{gfp}/+ mice demonstrated a small population of high Blimp-1 is expressing cells in the bone marrow (0.1-0.2%), spleen (0.4-0.6%) and lymph node (0.1%) (Figure 2). Further, phenotypic analysis of the GFP$^+$ cells indicated that they represented the previously defined Synd-1 high/B220 low ASC population as well as a previously poorly characterized Synd-1 low to negative phenotype (Figure 2, (Underhill *et al., (supra)*)). To confirm that these cells were ASC, GFP+ cells from *Blimp*^{gfp}/+ bone marrow and spleen were sorted and subjected to ELISpot analysis for Ig production. As can be seen in Figure 3, 75-100% of cells were Ig secreting cells (representative of 3 independent experiments). Moreover sorting of the GFP negative fraction, revealed a frequency of ASC of 0.001% (<1 per 100,000 cells), whereas the frequency of these cells in unsorted bone marrow was between 0.05-0.09% (50-90 per 100,000). Therefore the isolation of *Blimp*^{gfp} expressing ASC gives an enrichment of 100,000 fold over unsorted cells and provides a virtually definitive method to isolate these rare cells. In addition all Ig isotypes were represented in the GFP$^+$ ASC populations (Figure 3).

To further examine the production of ASC in *Blimp*^{gfp} /+ mice using the GFP reporter mice were injected with 2μg lipopolysaccharide (LPS) intravenously and analysed for *GFP* expression 1-7 days post-injection (LPS injection results in the polyclonal activation of mature B cells). LPS injection resulted in a dramatic increase in the numbers of splenic GFP$^+$ cells peaking at day 3 post-injection (~5% of total cells) before declining to steady state levels around day 7 (Figure 4A). Analysis of gated GFP$^+$ cells indicated that ASC

differentiation occurred in a synchronous wave with the appearance of Synd-1$^+$/B220$^+$ cells followed by Synd-1$^+$/B220$^-$ and finally a fraction of ASC become Synd-1$^-$/B220$^-$. This differentiation is also mirrored in the bone marrow where GFP$^+$ cells appear at day 4 post-injection as Synd-1$^+$/B220$^+$ cells that rapidly generate the Synd-1$^{+/-}$ /B220$^-$ steady state populations (Figure 4B). In summary, LPS treatment induces a wave of ASC differentiation that can be for the first time phenotypically defined using the *Blimp*^{gfp}/+ mouse stain.

EXAMPLE 4

Expression of *Blimp*-1 in ASC derived *in vitro*

A methodology was developed to quantitatively analyse the parameters affecting the commitment to and progression through the ASC lineage *in vitro*. This system involves the isolation of small resting B cells that are purified by Percoll gradient centrifugation and magnetic bead enrichment and cultured in the presence of a variety of stimuli that induce B cell proliferation and differentiation to ASC. These conditions include mimicking a T-dependent response using IL4 and anti-CD40 or a T-independent reaction using LPS. In addition IL5 can be titrated into these cultures to accelerate the rate of differentiation and anti-IgD (1.19) crosslinking carried out to activate an antigen specific response. Cultures were assayed on days 1-5 by flow cytometry to measure the frequency of *Blimp*^{gfp} and *Synd-1*$^+$ expressing cells. The number of ASC in the culture was determined by ELIspot.

Analysis of the time course of *Blimp*^{gfp} induction using CD40L/IL4/IL5 or LPS (Figure 5A) indicated that the first GFP$^+$ cells are observed in LPS cultures after 2 days. Thereafter, the numbers of positive cells increases until a peak at day 4 of approximately 50% GFP$^+$ cells. In contrast CD40L/IL4/IL5 treatment results in a delayed induction of fewer GFP expressing cells. Interestingly, whereas the majority of CD40L/IL4/IL5 induced GFP expressing cells are also *Synd-1*$^+$, LPS induces both *Synd-1*$^+$ and *Synd-1*$^-$ GFP expressing cells (Figure 5B). To determine if all the GFP$^+$ cells were actually ASC four fractions from LPS treated *Blimp*^{gfp} B cells were sorted (Figure 5C). ELIspot assays for IgM, IgG3 and IgG2b clearly show that all the ASC in the cultures are represented by

the GFP$^+$ fractions (A, B, C) and is not correlated with the levels of *Synd-1* expression. Moreover, the frequency of ASC did not vary between the GFP$^+$ populations (Figure 5C). Thus these data clearly show that whilst *Blimp*^{gfp} is a marker of the ASC fate, *Synd-1*$^+$ is only indicative of a sub-population of the ASC activity *in vitro* as it is *in vivo* (Figure 2). The regulation of *Blimp*^{gfp} and *Synd-1* expression was examined using the variety of stimuli outlined above. Interestingly, the frequency of GFP$^+$/Synd-1$^+$ and GFP$^+$/Synd-1$^-$ ASC formation can be modulated by different stimuli as outlined in Figure 5C. Thus, following *Blimp*^{gfp} expression provides a simple and definitive methodology to identify the modulators of ASC induction *in vitro* and *in vivo*.

Finally, a transplantation model was developed to demonstrate that *in vitro* derived *Blimp*^{gfp} positive ASC can be detected in the bone marrow or spleen of non-irradiated hosts 7 days after intravenous injection (Figure 6). Therefore, the *Blimp*^{gfp} allele provides a method to examine the effects of *in vitro* treatments of ASC on their survival, migration and functional properties *in vivo*.

EXAMPLE 5

Blimp-1 is required for embryogenesis

To produce homozygous *Blimp*$^{gfp/gfp}$ animals *Blimp*^{gfp}/+ individuals were intercrossed. Offspring from these crosses were genotyped at day 21 post-birth using *Blimp*-1 wild type and *Blimp*^{gfp} specific PCR primers. Whereas *Blimp*^{gfp}/+ mice were alive and healthy, no *Blimp*$^{gfp/gfp}$ individuals were identified indicating that *Blimp*-1 deficiency results in embryonic or early post-partum lethality (Figure 7). To examine more closely the stage at which *Blimp*$^{gfp/gfp}$ animals die, embryos produced from timed matings of *Blimp*$^{gfp/+}$ mice were examiner. These data indicate that *Blimp*$^{gfp/gfp}$ embryos are alive as late as embryonic stage E15.5. However, no viable older individuals have been documented. *Blimp*-1 is known to be widely expressed during embryogenesis, a finding that is supported by the analysis using the *Blimp*^{gfp} mouse.

EXAMPLE 6

Blimp-1 is essential for antibody production

To circumvent the embryonic lethality of *Blimp*$^{gfp/gfp}$ animals, and examine directly the importance of *Blimp*-1 in antibody production fetal liver stem cell reconstitution of lethally irradiated syngenic mice was used to produce adult mice that lack functional a functional Blimp-1 protein throughout the haematopoietic system. These *Blimp*$^{gfp/gfp}$ chimeric animals are healthy and contain relatively normal numbers of all the haematological lineages examined. *In vitro* analysis of the ASC population in these mice following stimulation with either LPS or CD40L/IL4 and IL5 revealed that the presence of GFP^{+} *Blimp* deficient cells that were predominantly synd-1^{+} (Figure 8A). Importantly, these cells failed to secrete antibody as assessed by ELIspot assay (Figure 8B). Therefore, the *Blimp*^{gfp} the mouse model described here not only provides a definitive tool to isolate ASC but enables the identification of the population of *Blimp-1* expressing cells from homozygous mutant *Blimp*$^{gfp/gfp}$ splenocytes, thereby greatly facilitating the analysis of the mechanism underlying the phenotype of Blimp-1 deficiency.

EXAMPLE 7

Expression of *Blimp-1* in other haematopoietic lineages

The *Blimp*^{gfp} reporter system has also enabled for the first time define the expression pattern of *Blimp*-1 in haematopoiesis. As stated above analysis of the lymphoid organs of *Blimp*^{gfp} mice revealed that the GFP high producing populations are almost exclusively ASC. However, lower level GFP producing cells were also apparent.

Blimp-1 has been reported to be expressed by human and mouse macrophages and granulocytes. Flow cytometric analysis of blood monocytic cells and and bone marrow derived macrophages cultures in the presence of MCSF-1 revealed clear *Blimp*^{gfp} expression in these cell types (Figure 9). However, no GFP fluorescence was observed in granulocytes. *In vivo* isolated dendritic cells in contrast lack *Blimp*-1 mRNA expression. Similarly, plasmacytoid and conventional dendritic cells derived from the culture of bone

marrow cells with flt3L lack *Blimp*^{gfp} fluorescence. However, the *ex vivo* activation of sorted dendritic cells or the *in vitro* activation of the flt3L cultures by CpG DNA results in *Blimp*-1 expression predominantly conventional dendritic cells (Figure 10).

Analysis of thymus and resting spleen from *Blimp*$^{gfp/+}$ mice demonstrated that *Blimp*-1 is not expressed during T cell development. However, a small population of *Blimp*^{gfp} expressing T cells were present in lymph nodes. As these cells could represent the small population of activated T cells present we have stimulated lymph node T cells *in vitro* with an anti-CD3 monoclonal antibody in the presence or absence of concanavalin A, conditions known to strongly activate T cells. In support of the *in vivo* analysis, *in vitro* activated T cells expressed *Blimp*^{gfp} (Figure 12C).

Examination of the NK lineage in *Blimp*^{gfp} mice revealed that unlike the other haematopoietic lineages examined NK cells constitutively express *Blimp*-1. NK cells were identified from blood, spleen and bone marrow as NK1.1$^+$/CD122$^+$ cells and demonstrated to be uniformly GFP$^+$ (Figure 12A). This expression was maintained *in vitro* as mature NK cells cultured in the presence of IL15 are GFP$^+$ and can be further induced by cytokines such as IL21 or IL12/IL18 that induce NK cell terminal differentiation (Figure 12B). The expression of *Blimp*-1 in NK cells was also confirmed by Western blotting with a Blimp-1 specific monoclonal antibody.

In summary, the *Blimp*^{gfp} reporter mouse has revealed that Blimp-1 is induced in the late stages of a variety of haematopoietic lineages thereby providing a method of identifying the regulators of the maturation of these cell types. Importantly, the relatively lower production levels of GFP in non-B lymphoid cell types does not interfere with the isolation of homogenous populations of ASC.

EXAMPLE 8

Examining the role of Blimp-1 in cancer using the *Blimp*^{gfp} mouse

In addition to its utility in examining ASC differentiation, the *Blimp*^{gfp} reporter mouse can be used to examine the malignant transformation of this cell type. Tumors of ASC, designated plasmacytomas in mice and multiple myeloma in humans, are specifically and frequently elicited in Eμ-v-*abl* transgenic mice (Rosenbaum *et al., (supra)*), which express the v-*abl* oncogene in the B cell lineage, under the control of the IgH intronic enhancer. These mice were crossed with *Blimp*^{gfp} mutant mice, to determine the affect of loss of one or both copies of the *Blimp-1* gene on latency and incidence of tumors. Two outcomes are envisaged: Blimp-1, by inducing the plasma cell differentiation program, might be required to open the window of opportunity for v-*abl* transformation. This transgene induces only plasmacytomas, despite expression in earlier B cells (Rosenbaum *et al., (supra)*). Therefore, loss of functional *Blimp-1* alleles would be predicted to decrease tumor incidence or increase latency. Alternatively, as a large proportion of v-*abl*-induced plasmacytomas also bear a rearranged and activated *c-myc* gene, it may be that Myc is an essential cooperating activity in the transformation (Rosenbaum *et al., (supra)*). Blimp-1 is believed normally to repress *c-myc* expression during terminal ASC differentiation (Lin *et al., Science 276:596*, 1997). In this scenario, loss of functional Blimp-1 should allow continued *c-myc* expression, which may accelerate plasmacytoma development.

If Blimp-1 is indeed playing a role in ASC tumorogenesis, the *Blimp*^{gfp} reporter strain provides, therefore, a useful animal model to deterne the effects of inhibiting/inducing Blimp-1 on tumor progression.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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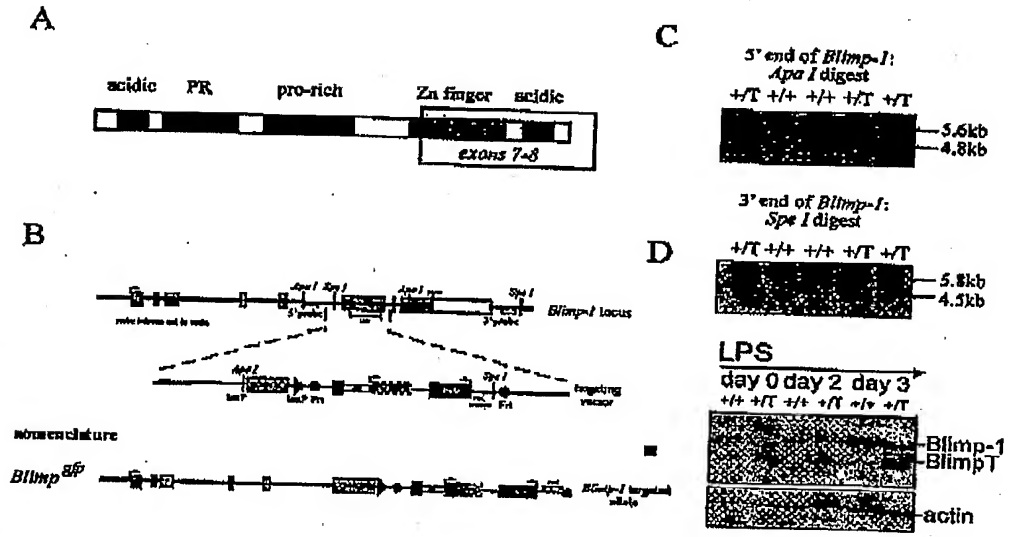

A
acidic
PR
pro-rich
Zn finger
acidic
exons 7-8
B
Blimp-1 locus
nomenclature
C
3' end of Blimp-1:
Apa I digest
+/T +/+ +/+ +/T +/T
5.6kb
4.8kb
D
3' end of Blimp-1:
Spe I digest
+/T +/+ +/+ +/T +/T
5.8kb
4.5kb
LPS
day 0 day 2 day 3
Blimp-1
BlimpT
actin

FIGURE 1

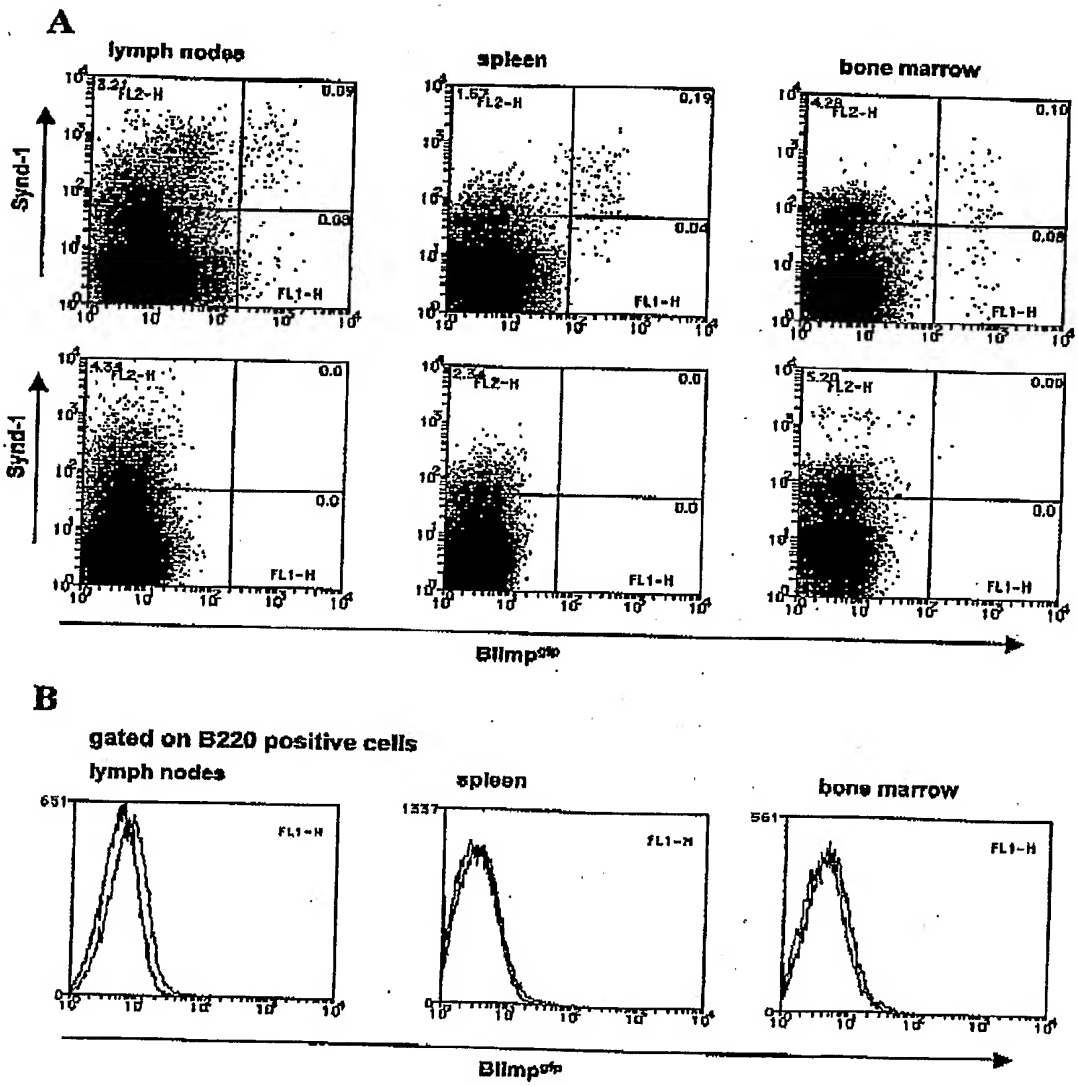
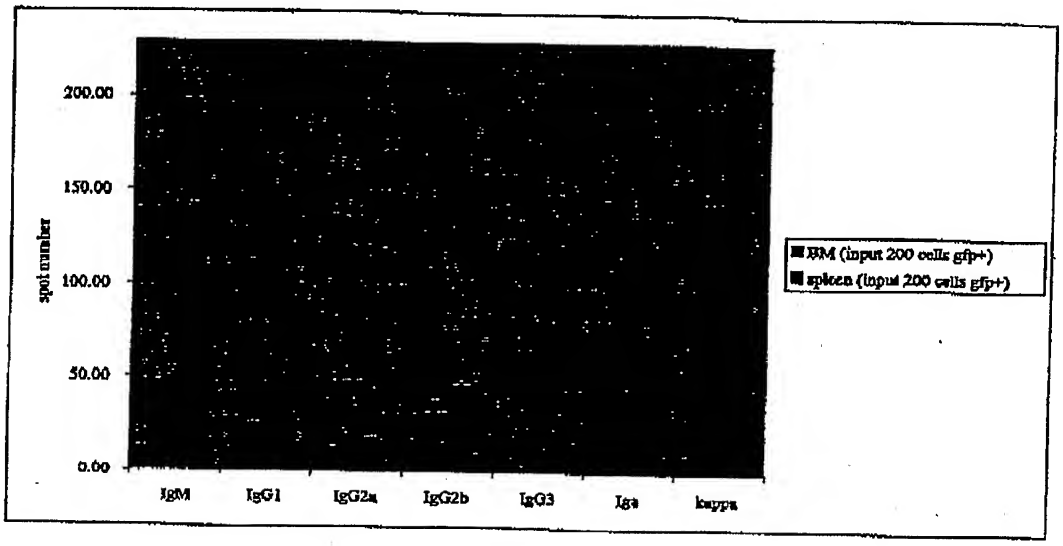

A
lymph nodes
spleen
bone marrow
Synd-1
FL2-H
FL1-H
8.2
0.09
0.03
1.67
0.19
0.04
4.28
0.10
0.08
4.34
0.0
2.34
5.20
0.00
Blimp^gfp
B
gated on B220 positive cells
lymph nodes
spleen
bone marrow
651
1337
561
FL1-H
Blimp^gfp

FIGURE 2

A


200.00
150.00
100.00
50.00
0.00
spot number
IgM
IgG1
IgG2a
IgG2b
IgG3
Iga
kappa
BM (input 200 cells gfp+)
spleen (input 200 cells gfp+)

B

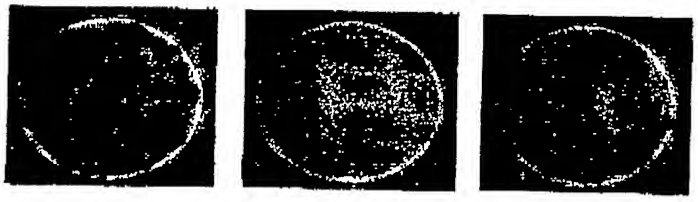


FIGURE 3

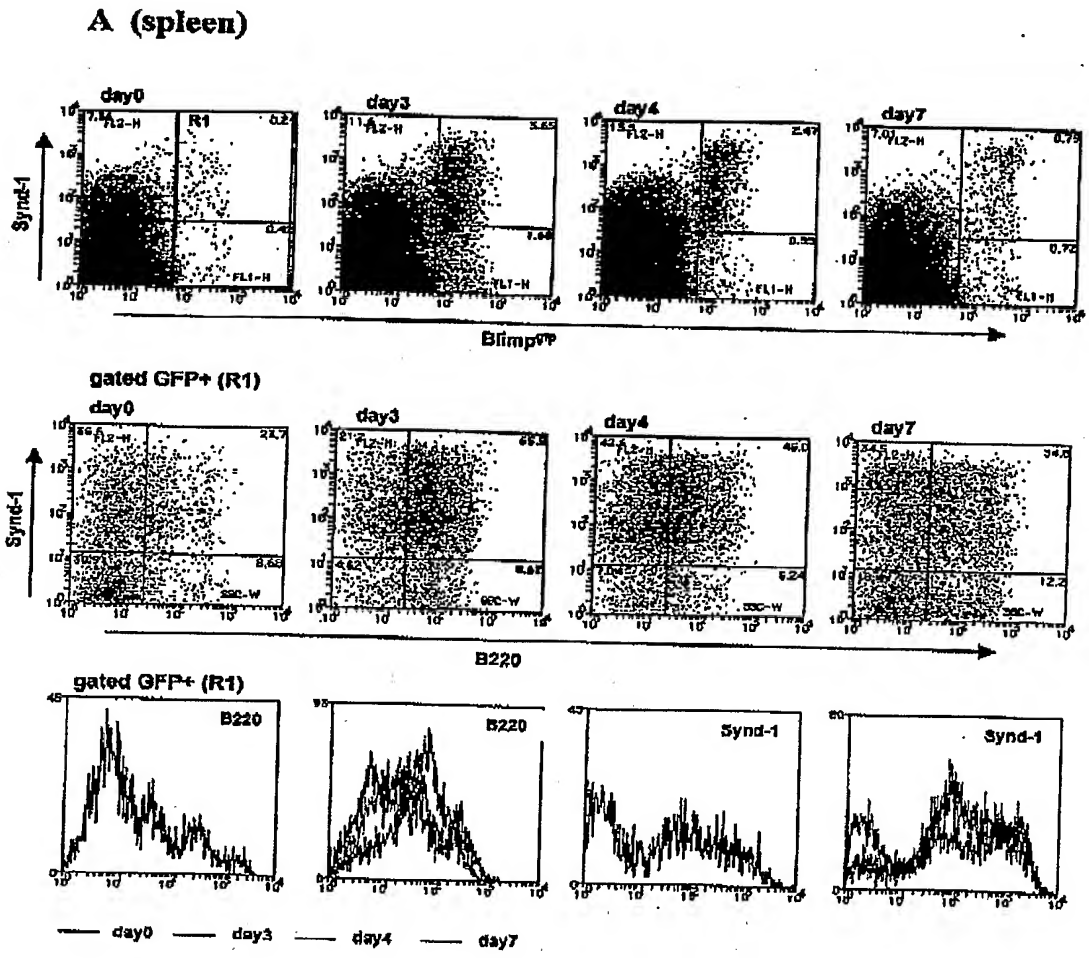

A (spleen)
day0
day3
day4
day7
Synd-1
Blimp^{GFP}
gated GFP+ (R1)
B220
Synd-1
day0
day3
day4
day7

FIGURE 4

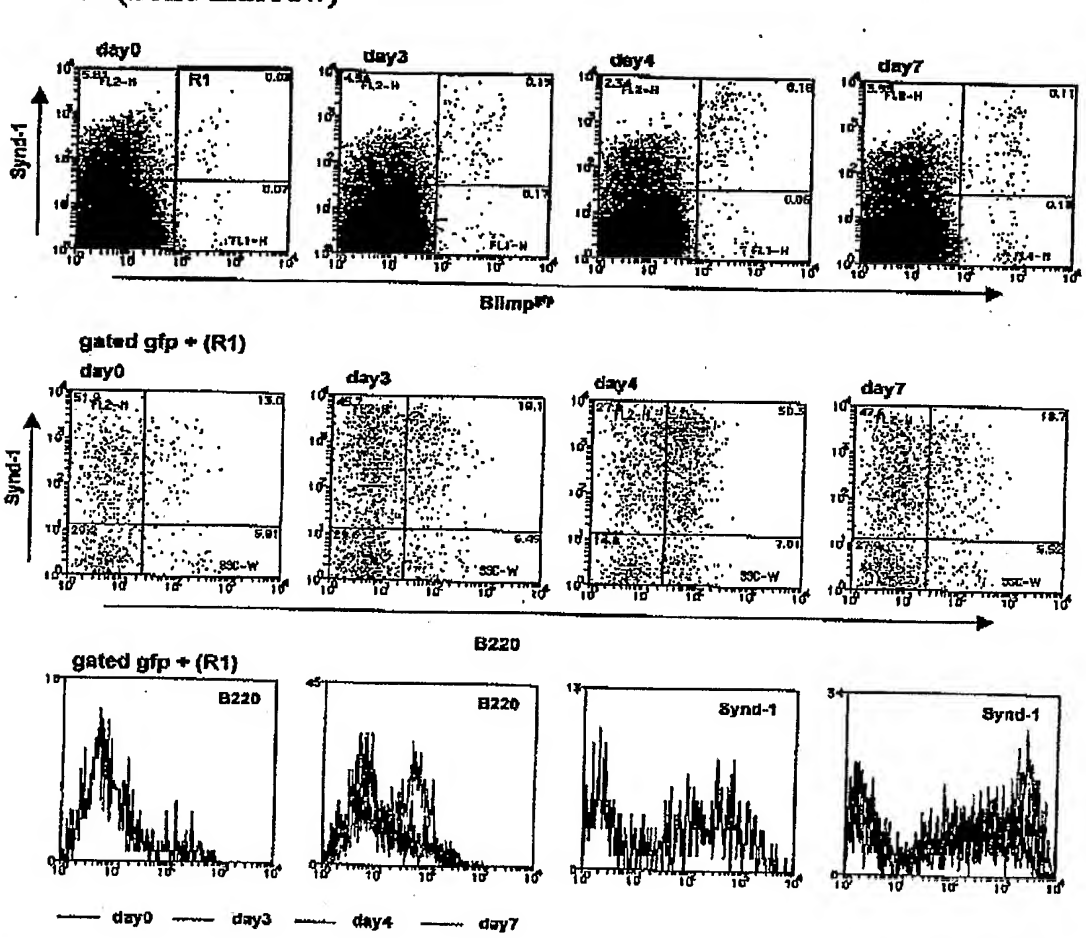
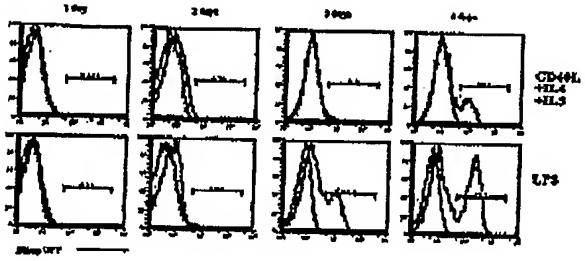
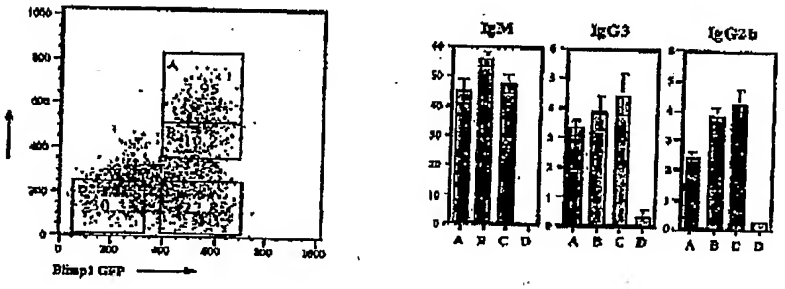


FIGURE 4 cont.

A



B



C

FIGURE 5

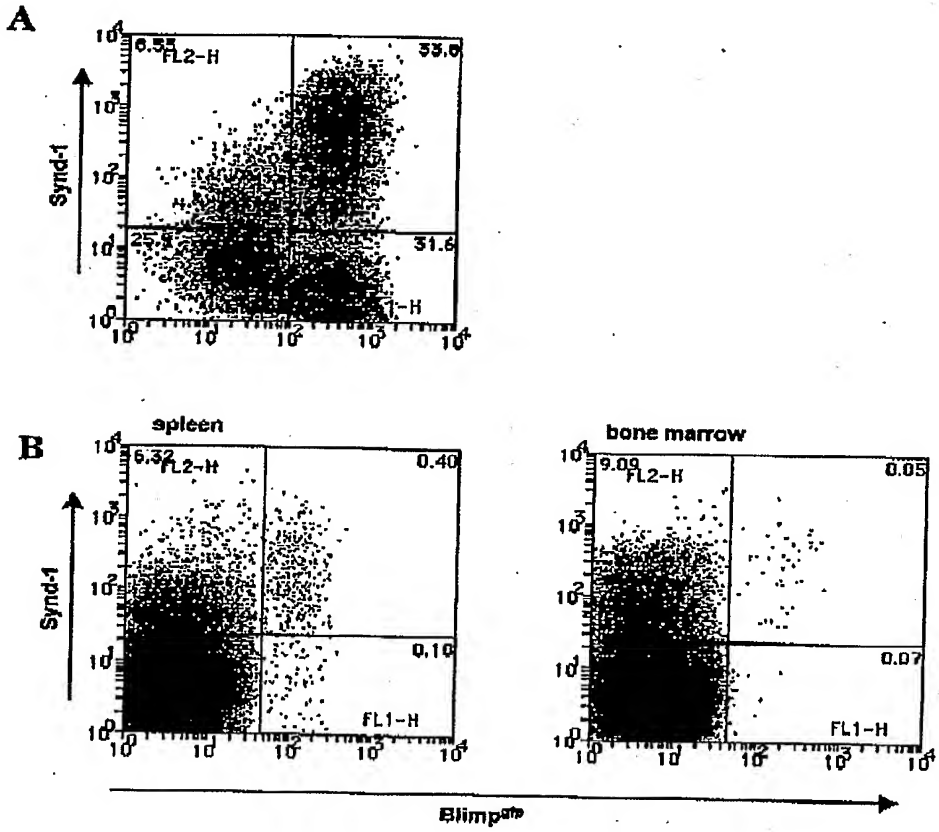

A
6.55
FL2-H
33.6
Synd-1
25.8
31.6
FL1-H
B
spleen
6.32
FL2-H
0.40
Synd-1
0.10
FL1-H
bone marrow
9.09
FL2-H
0.05
0.07
FL1-H
Blimp1

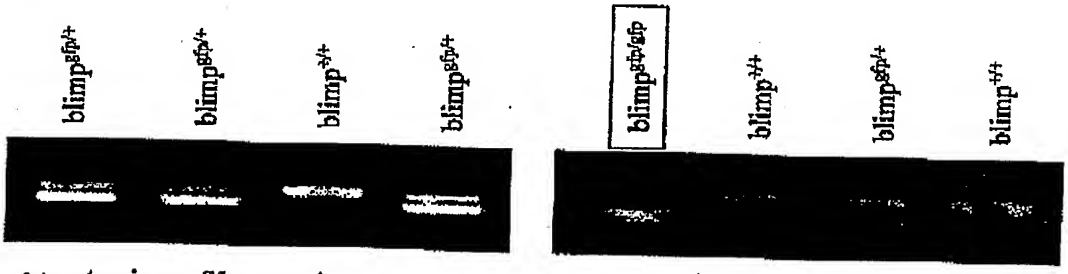
FIGURE 6

A

$blimp^{gfp/+}$ x $blimp^{gfp/+}$

	$blimp^{+/+}$	$blimp^{gfp/+}$	$blimp^{gfp/gfp}$
# of mice born	19	25	–

B



genotyping of born mice

genotyping of E9.5 embryos

FIGURE 7

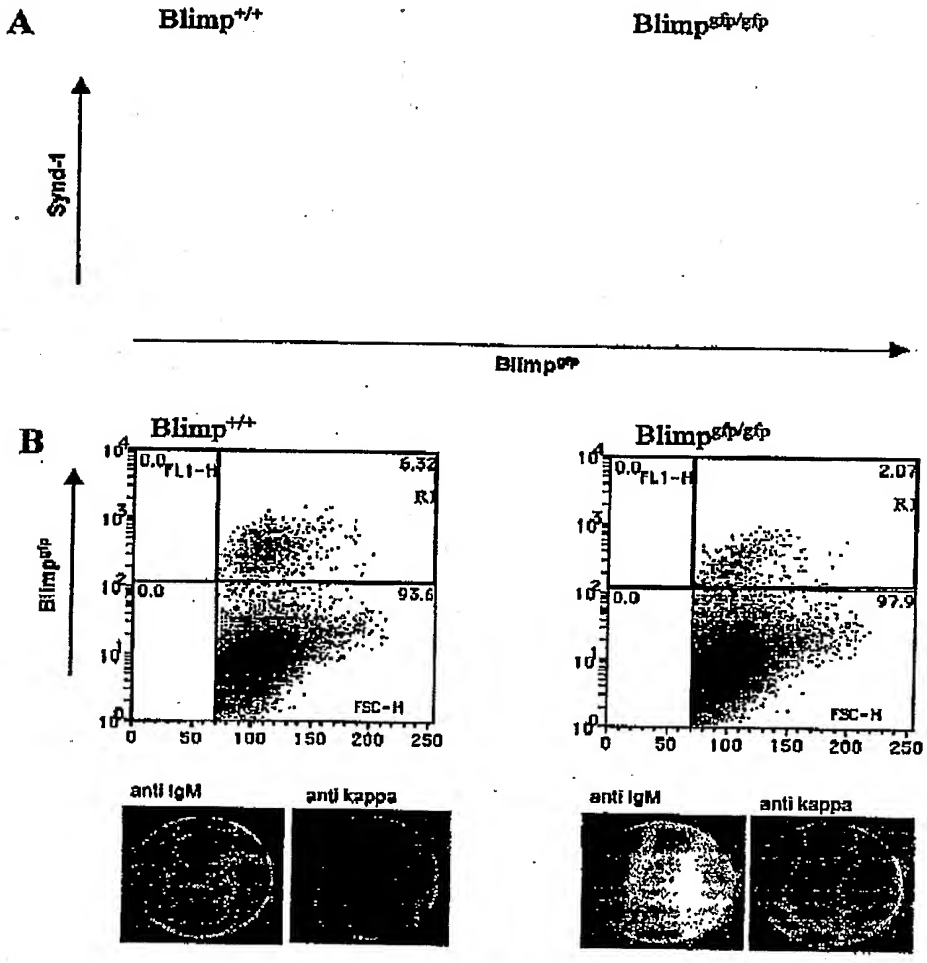

A
Blimp+/+
Blimpgfp/gfp
Synd-1
Blimpgfp
B
Blimp+/+
Blimpgfp/gfp
Blimpgfp
0.0
FL1-H
6.32
R1
93.6
FSC-H
2.07
97.9
anti IgM
anti kappa

FIGURE 8

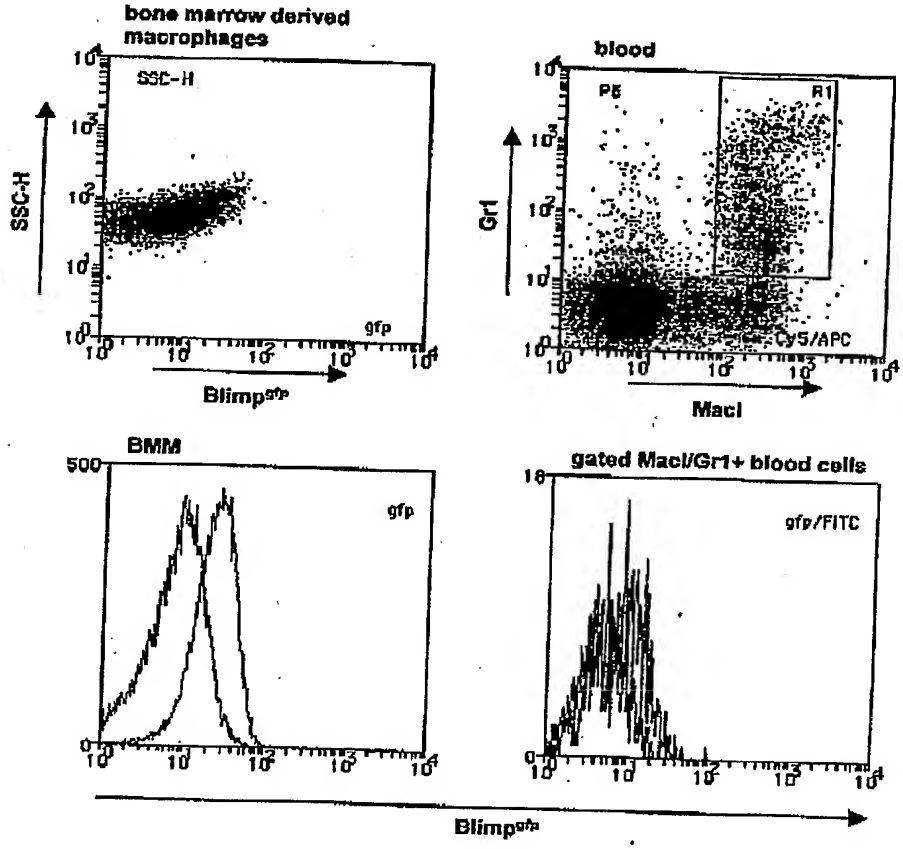

bone marrow derived macrophages
SSC-H
gfp
Blimp^gfp
blood
P5
R1
Gr1
Cy5/APC
Mac1
BMM
gfp
gated Mac1/Gr1+ blood cells
gfp/FITC
Blimp^gfp

FIGURE 9

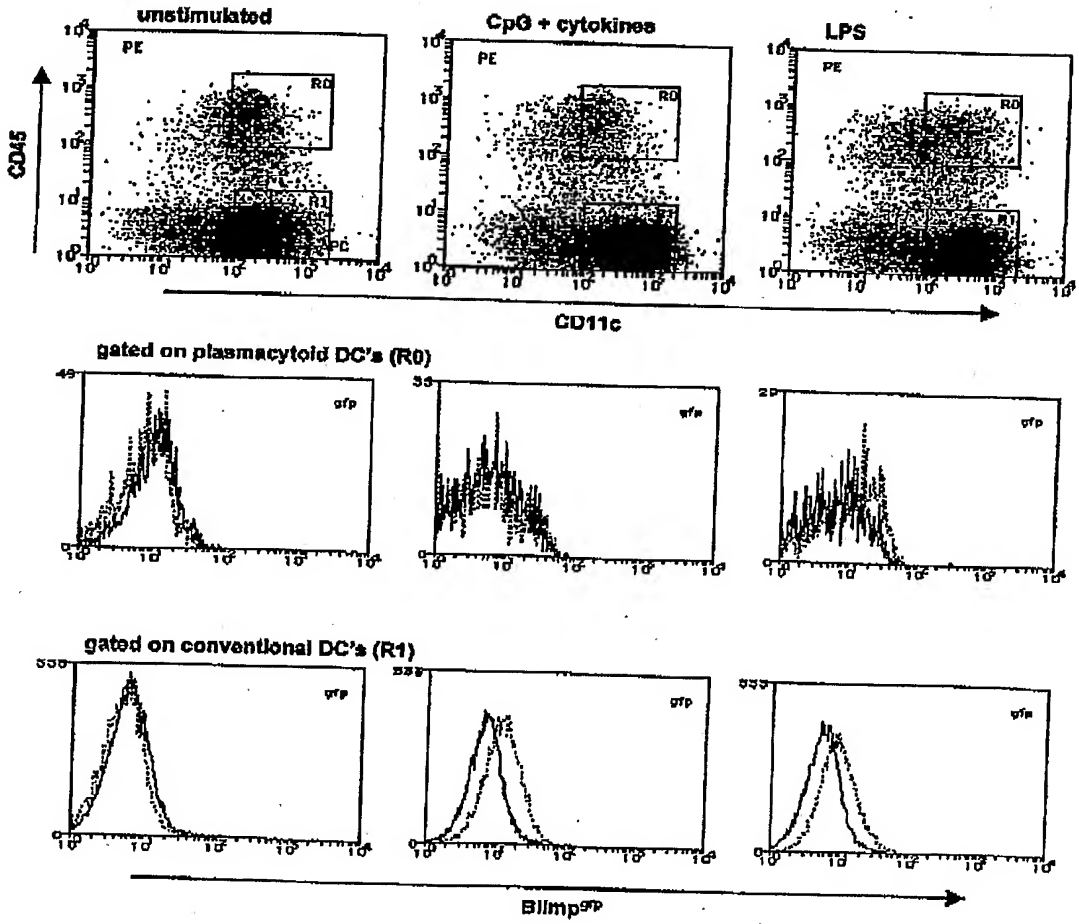

unstimulated
CpG + cytokines
LPS
PE
R0
R1
CD45
CD11c
gated on plasmacytoid DC's (R0)
gated on conventional DC's (R1)
gfp
Blimp^gfp

FIGURE 10

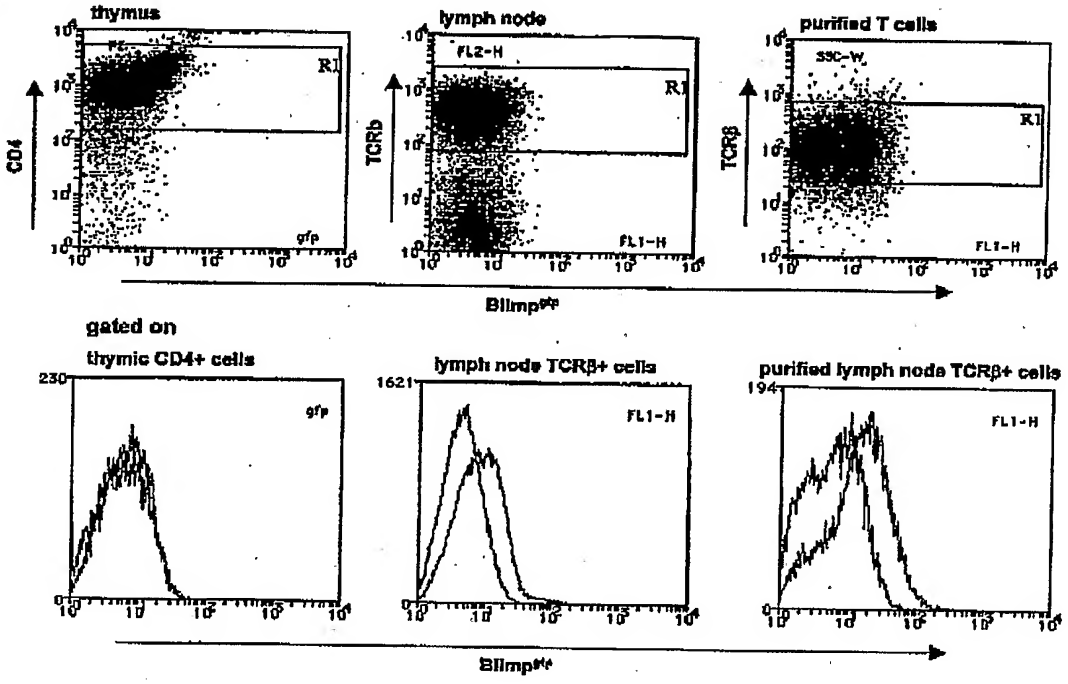

thymus
lymph node
purified T cells
CD4
TCRb
TCRβ
R1
gfp
FL2-H
FL1-H
SSC-W
Blimp^gfp
gated on
thymic CD4+ cells
lymph node TCRβ+ cells
purified lymph node TCRβ+ cells
230
1621
194

FIGURE 11

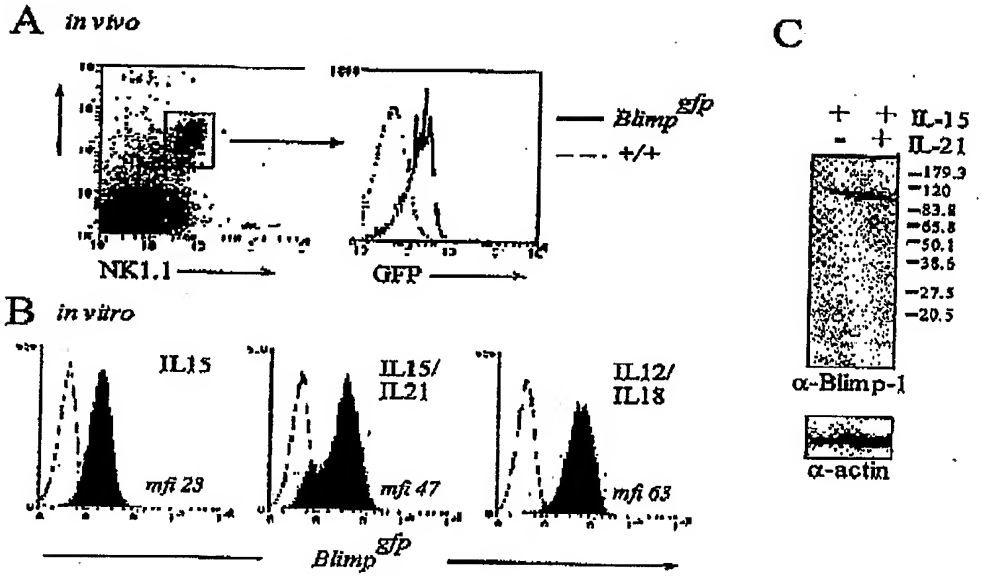

A in vivo
Blimp gfp
+/+
NK1.1
GFP
B in vitro
IL15
IL15/
IL21
IL12/
IL18
mfi 23
mfi 47
mfi 63
Blimp gfp
C
+ + IL-15
- + IL-21
179.3
120
83.8
65.8
50.1
36.6
27.5
20.5
α-Blimp-1
α-actin

FIGURE 12

ggggggaagagtagtcagtcgctcgctcactcgctcgctcgcacagacactgctgcagtga
cactcggccctccagtgtcgcggagacgcaagagcagcgcgcagcacctgtccgcccgga
gcgagcccggcccgcggccgtagaaaaggagggaccgccgaggtgcgcgtcagtactgct
cagcccggcagggacgcgggaggatgtggactgggtggac**ATGAGAGAGGCTTATCTCAG**
ATGTTGGATCTTCTCTTGGAAAAACGTGTGGGTACGACCTTGCCAAAGGCTGCATTTTAA
AACCGTGCTTCTTCAAGGCAGTCTACTTTACACGGCTTTGGACTCTTACTCAACTGTACA
AGCTGCCCCCAAGTCTAGCTCCGGCTCCGTGAAGTTTCAAGGACTGGCAGAGACTGGGAT
CATGAAAATGGACATGGAGGACGCTGATATGACTTTGTGGACAGAGGCCGAGTTTGAAGA
GAAGTGTACATACATAGTGAACGACCACCCCTGGGATTCCGGCGCTGACGGGGGTACTTC
TGTTCAAGCCGAGGCATCCTTACCAAGGAACCTGCTTTTCAAGTATGCTGCCAACAACAG
CAAAGAGGTTATTGGCGTGGTAAGTAAGGAGTACATACCGAAGGGAACACGCTTTGGACC
CCTCATCGGTGAAGTCTACACTAATGACACAGTTCCCAAGAATGCCAACAGGAAGTATTT
TTGGCGGATCTATTCCAGAGAGGAGTTCCACCACTTCATTGATGGCTTTAATGAGGAGAA
AAGCAACTGGATGCGCTACGTGAATCCAGCTCACTCTGCCCGGGAGCAAAACCTGGCTGC
CTGTCAGAACGGGATGAACATCTACTTCTACACTATTAAGCCTATCCCTGCCAACCAGGA
ACTTCTTGTGTGGTATTGTCGGGACTTTGCGGAGAGGCTCCACTACCCTTATCCTGGAGA
GCTCACAGTGATAAATCTCACACAAACGGAAAGCAACCCAAAGCAATACAGTAGTGAGAA
AAATGAACTCTACCCAAAGAGTGTCCCCAAGAGAGAGTACAGCGTGAAAGAAATTCTAAA
ACTGGACTCCAATCCCTCCAAAAGGAAGGACATCTACCGTTCCAACATTTCACCCTTCAC
TTTAGAAAAGGACATGGATGGCTTTCGGAAAAATGGGAGCCCCGACATGCCCTTCTACCC
TCGGGTGGTTTATCCTATCCGGGCACCTCTGCCAGAAGACTTTTTGAAAGCGTCCCTGGC
CTATGGGATGGAGAGACCCACCTACATAACTCACAGTCCCCTTCCGTCTTCCACAACTCC
AAGTCCCCCTGCGAGCAGCAGCCCGGAGCAGAGCCTTAAGAGCTCCAGCCCCCACAGCAG
CCCGGGAAACACGGTGTCACCCCTGGCGCCAGGCCTCCCAGAACACCGGGACTCCTACTC
CTACTTGAATGTTTCCTATGGTTCCGAGGGCCTGGGCTCCTACCCTGGCTATGCACCTGC
CCCCCACCTCCCACCAGCTTTCATTCCTTCTTACAATGCTCACTACCCCAAGTTCCTGTT
GCCACCGTACGGCATTAGTTCCAATGGCTTGAGCACCATGAACAACATCAATGGTATCAA

FIGURE 13

CAACTTCAGCCTCTTCCCTAGGTTGTATCCCGTCTACAGTAACCTCCTTAGTGGCAGCAG
CCTGCCTCATCCCATGCTCAATCCAGCTTCCCTACCGAGTTCCCTGCCTACCGATGGAGC
CCGGAGGCTGCTTCCACCGGAGCACCCCAAAGAGGTGCTTATCCCAGCACCCCACAGTGC
CTTCTCCCTTACCGGGGCTGCAGCCAGCATGAAGGACGAGAGTAGTCCCCCCAGCGGATC
TCCAACGGCGGGAACTGCAGCCACGTCAGAACACGTGGTACAACCCAAAGCTACCTCATC
AGTGATGGCGGCCCCCAGCACTGACGGAGCCATGAATCTCATTAAAAACAAACGAAACAT
GACTGGTTACAAGACTCTTCCTTACCCTCTGAAGAAACAGAATGGCAAGATCAAGTATGA
GTGCAATGTCTGTGCCAAGACGTTCGGTCAGCTCTCCAACCTGAAGGTCCACCTGAGAGT
GCACAGTGGAGAACGGCCTTTCAAGTGCCAGACCTGCAACAAGGGTTTTACTCAGCTCGC
CCACCTGCAGAAACACTACTTGGTACACACAGGAGAGAAGCCACATGAGTGCCAGGTCTG
CCACAAGAGATTTAGCAGCACAAGCAATCTCAAGACCCACCTTCGATTGCATTCTGGAGA
AAAACCTTACCAATGTAAGGTGTGCCCTGCCAAGTTTACGCAATTTGTGCACCTGAAGCT
GCACAAGCGACTGCATACCCGGGAGCGGCCTCACAAGTGTGCCCAGTGTCACAAGAGCTA
CATCCATCTCTGCAGCCTCAAGGTCCACCTGAAGGGCAACTGCCCTGCGGGCCCAGCTGC
TGGGCTGCCTTTGGAGGATCTGACCCGAATCAATGAAGAAATTGAGAGGTTCGACATCAG
CGACAATGCAGACCGTCTTGAGGACATGGAGGACAGTGTCGATGTGACCTCCATGGTGGA
GAAGGAGATTCTAGCTGTGGTCAGAAAAGACAAAGAAGAAACCAGTCTGAAAGTGTCTTT
GCAAAGAAACATGGGGAACGGCCTCCTCTCCTCAGGGTGCAGCCTCTATGAGTCATCGGA
CCTGTCCCTCATGAAGTTGCCTCACAGCAACCCACTACCTCTGGTGCCTGTAAAGGTCAA
ACAAGAAACAGTTGAACCGATGGATCCTTAAgattttcagaaaataagtgtttcgtgttg
cttcttagggtatggcttggtgaatcagggtgcctttagcaaattgcttgtacatgactc
cagatctgcaaagctccgctggcaccgggtgcttccctgcacctctctggaattaaagaa
ggactccaatgttaccaaaatctcagggcataaatgaggcaaagactcactatatataca
tatatacatatatacatattataaatatatatatacttatttacagccatgtctatatat
ttgaacctgtgtattttgaatatttgtgtggatatgtttgcatagcgccttcctattact
aaaactattgcctagccataattatttttttcaatgataattcttcataatttattataca
gtttatctttcaaaaagcaataattaaagaagtttacaatgactggaaagattctttgta

FIGURE 13 cont.

atttgagtataaatgttgtatctttgtcctgtggccattctttgtagataatttctgcac
atctgtttaaatgcctgagacttagaagatagctctgtgatttcaggcaacctttctcta
tgataatgctttaaaatgaggttttgatattgccaaagtcatgtggttggtgtgttaact
cagaagatcacacaatctgagtgacattctctaagttggggatacatgtgcagaattgct
cagcaataatttgaggggaaggaagaagaaaaatattttatgtttcagaatgatggtttg
gttttcctcctcctagtcacaattttaccaaacagtgacaggaaggctttgccaacctgt
ctcccaatgtcacatgaccattctgagtggccatatgactttggcatccctgggtgttat
ctgaaaatgtgaagaagataaaaaagccgtgttcagaagatctgtcgtaaagcacagatg
ttgtgtgtgtgtgtgtgtgggttgggggtttgagtctggctgtcattttgctgttggct
tgtttttgtttttttaatatcaaaattgcacaaagctggtgccctaccaagaaggatttg
atatagaaaggctcaggccacacttaaaatacaagcaagcaaagagaacagaaaaaata
aaagtaaaaacgggtattcttatcatcttaggttaagcgggtaatgaacattcctgtccc
caacgcatcaactgtattgtatctgtaaaactcagcttttctcagtatttgtgtttttgc
attgtataattaacttaattaaagatgaaagggcattgcaaaagtgttcaacaattacct
cattgagtgtatccagtagaagtgcaggaattaatgtcgtatctcatgagttgctaccca
gctgagcgtgtgtgcttccaaatggtaggctgggtggttcggtcctgtattctcctaagc
ccaaaggttacctgttggtgttcaaggtgtaataaagaatgctgtatatttatgaaccta
tttataccagtataccatgtgtatatatgatatatttataaccacttaaattgtgagcca
agccatgtaaaagaacctatttttcctaagagcaaaaagaatctctctgaagttttgctt
aaaactccatgacctcgctatgactttggtgcttgggcaccaccctgcctactaccagag
agcagagcacctcagtgcagaggtgagggtgtgtagcatcttgggatggatagaaacacc
acaccatccagtcgcatttgatggccttgctacatgtgtgtcagttgggtcacagaataa
aaatcatttttctatttctgctctcctcttcctcttcctcttcttcttcctctttctctc
cctcctctagaaccctgactcatgctcactgctcagtctgatgcttaccttagagttttg
tatatatagatcaacttacaaagagggaaaacttcagatcctctgggggaaacccaagag
ccttactgacctgttgctgtgactagctagatgggtttctctttaccttccaaggatcaa
aaccagagattccacacatgctagcaagcaagcaagctgtcactgggctgcagccccaac

FIGURE 13 cont.

aagactgacatttctggatgcatctgtatttgagaaaaatactcacttaattgtaggtta
accaaagcatgacctgacattgacaccaaatacaaatacgatttctttgcagtgaacttg
ggttgttttcctcctgtgcttttcttgtgttgggaggatttttacaaggacaattgcttt
tcttgccatctgtcttcctcttaggcctcttacatgagagtgttgagcccacaatgaaca
gtggttggttggttggttggtttgtttgtttgtttttttcctcagagttaaaactga
ccaaagttattggcttttttactttgctagaacaacaaactatcttatgtttacgtactgg
tttacattgttatttatgtgcaaattgtcaaaatgtaaattaaaatataaatgttcatgc
t

FIGURE 13 cont.

MREAYLRCWIFSWKNVWVRPCQRLHFKTVLLQGSLLYTALDSYSTVQAAPKSSSGSVKFQ
GLAETGIMKMDMEDADMTLWTEAEFEEKCTYIVNDHPWDSGADGGTSVQAEASLPRNLLF
KYAANNSKEVIGVVSKEYIPKGTRFGPLIGEVYTNDTVPKNANRKYFWRIYSREEFHHFI
DGFNEEKSNWMRYVNPAHSAREQNLAACQNGMNIYFYTIKPIPANQELLVWYCRDFAERL
HYPYPGELTVINLTQTESNPKQYSSEKNELYPKSVPKREYSVKEILKLDSNPSKRKDIYR
SNISPFTLEKDMDGFRKNGSPDMPFYPRVVYPIRAPLPEDFLKASLAYGMERPTYITHSP
LPSSTTPSPPASSSPEQSLKSSSPHSSPGNTVSPLAPGLPEHRDSYSYLNVSYGSEGLGS
YPGYAPAPHLPPAFIPSYNAHYPKFLLPPYGISSNGLSTMNNINGINNFSLFPRLYPVYS
NLLSGSSLPHPMLNPASLPSSLPTDGARRLLPPEHPKEVLIPAPHSAFSLTGAAASMKDE
SSPPSGSPTAGTAATSEHVVQPKATSSVMAAPSTDGAMNLIKNKRNMTGYKTLPYPLKKQ
NGKIKYECNVCAKTFGQLSNLKVHLRVHSGERPFKCQTCNKGFTQLAHLQKHYLVHTGEK
PHECQVCHKRFSSTSNLKTHLRLHSGEKPYQCKVCPAKFTQFVHLKLHKRLHTRERPHKC
AQCHKSYIHLCSLKVHLKGNCPAGPAAGLPLEDLTRINEEIERFDISDNADRLEDMEDSV
DVTSMVEKEILAVVRKEKEETSLKVSLQRNMGNGLLSSGCSLYESSDLSLMKLPHSNPLP
LVPVKVKQETVEPMDP

FIGURE 14

gaattccgggaagccagacggttaacacagacaaagtgctgccgtgacactcggccctcc
agtgttgcggagaggcaagagcagcgaccgcgcacctgtccgcccggagctgggacgcgc
gcccgggcggccggacgaagcgaggagggaccgccgaggctgcccccaagtgtaactcca
gcactgtgaggtttcagggattggcagaggggaccaaggggac**ATGAAAATGGACATGGA**
GGATGCGGATATGACTCTGTGGACAGAGGCTGAGTTTGAAGAGAAGTGTACATACATTGT
GAACGACCACCCCTGGGATTCTGGTGCTGATGGCGGTACTTCGGTTCAGGCGGAGGCATC
CTTACCAAGGAATCTGCTTTTCAAGTATGCCACCAACAGTGAAGAGGTTATTGGAGTGAT
GAGTAAAGAATACATACCAAAGGGCACACGTTTTGGACCCCTAATAGGTGAAATCTACAC
CAATGACACAGTTCCTAAGAACGCCAACAGGAAATATTTTTGGAGGATCTATTCCAGAGG
GGAGCTTCACCACTTCATTGACGGCTTTAATGAAGAGAAAAGCAACTGGATGCGCTATGT
GAATCCAGCACACTCTCCCCGGGAGCAAAACCTGGCTGCGTGTCAGAACGGGATGAACAT
CTACTTCTACACCATTAAGCCCATCCCTGCCAACCAGGAACTTCTTGTGTGGTATTGTCG
GGACTTTGCAGAAAGGCTTCACTACCCTTATCCCGGAGAGCTGACAATGATGAATCTCAC
ACAAACACAGAGCAGTCTAAAGCAACCGAGCACTGAGAAAAATGAACTCTGCCCAAAGAA
TGTCCCAAAGAGAGAGTACAGCGTGAAAGAAATCCTAAAATTGGACTCCAACCCCTCCAA
AGGAAAGGACCTCTACCGTTCTAACATTTCACCCCTCACATCAGAAAAGGACCTCGATGA
CTTTAGAAGACGTGGGAGCCCCGAAATGCCCTTCTACCCTCGGGTCGTTTACCCCATCCG
GGCCCCTCTGCCAGAAGACTTTTTGAAAGCTTCCCTGGCCTACGGGATCGAGAGACCCAC
GTACATCACTCGCTCCCCCATTCCATCCTCCACCACTCCAAGCCCCTCTGCAAGAAGCAG
CCCCGACCAAAGCCTCAAGAGCTCCAGCCCTCACAGCAGCCCTGGGAATACGGTGTCCCC
TGTGGGCCCCGGCTCTCAAGAGCACCGGGACTCCTACGCTTACTTGAACGCGTCCTACGG
CACGGAAGGTTTGGGCTCCTACCCTGGCTACGCACCCCTGCCCCACCTCCCGCCAGCTTT
CATCCCCTCGTACAACGCTCACTACCCCAAGTTCCTCTTGCCCCCCTACGGCATGAATTG
TAATGGCCTGAGCGCTGTGAGCAGCATGAATGGCATCAACAACTTTGGCCTCTTCCCGAG
GCTGTGCCCTGTCTACAGCAATCTCCTCGGTGGGGGCAGCCTGCCCCACCCCATGCTCAA
CCCCACTTCTCTCCCGAGCTCGCTGCCCTCAGATGGAGCCCGGAGGTTGCTCCAGCCGGA
GCATCCCAGGGAGGTGCTTGTCCCGGCGCCCCACAGTGCCTTCTCCTTTACCGGGGCCGC

FIGURE 15

CGCCAGCATGAAGGACAAGGCCTGTAGCCCCACAAGCGGGTCTCCCACGGCGGGAACAGC
CGCCACGGCAGAACATGTGGTGCAGCCCAAAGCTACCTCAGCAGCGATGGCAGCCCCCAG
CAGCGACGAAGCCATGAATCTCATTAAAAACAAAAGAAACATGACCGGCTACAAGACCCT
TCCCTACCCGCTGAAGAAGCAGAACGGCAAGATCAAGTACGAATGCAACGTTTGCGCCAA
GACTTTCGGCCAGCTCTCCAATCTGAAGGTCCACCTGAGAGTGCACAGTGGAGAACGGCC
TTTCAAATGTCAGACTTGCAACAAGGGCTTTACTCAGCTCGCCCACCTGCAGAAACACTA
CCTGGTACACACGGGAGAAAAGCCACATGAATGCCAGGTCTGCCACAAGAGATTTAGCAG
CACCAGCAATCTCAAGACCCACCTGCGACTCCATTCTGGAGAGAAACCATACCAATGCAA
GGTGTGCCCTGCCAAGTTCACCCAGTTTGTGCACCTGAAACTGCACAAGCGTCTGCACAC
CCGGGAGCGGCCCCACAAGTGCTCCCAGTGCCACAAGAACTACATCCATCTCTGTAGCCT
CAAGGTTCACCTGAAAGGGAACTGCGCTGCGGCCCCGGCGCCTGGGCTGCCCTTGGAAGA
TCTGACCCGAATCAATGAAGAAATCGAGAAGTTTGACATCAGTGACAATGCTGACCGGCT
CGAGGACGTGGAGGATGACATCAGTGTGATCTCTGTAGTGGAGAAGGAAATTCTGGCCGT
GGTCAGAAAAGAGAAAGAAGAAACTGGCCTGAAAGTGTCTTTGCAAAGAAACATGGGGAA
TGGACTCCTCTCCTCAGGGTGCAGCCTTTATGAGTCATCAGATCTACCCCTCATGAAGTT
GCCTCCCAGCAACCCACTACCTCTGGTACCTGTAAAGGTCAAACAAGAAACAGTTGAACC
AATGGATCCTTAAgattttcagaaaacacttattt

FIGURE 15 cont.

MKMDMEDADMTLWTEAEFEEKCTYIVNDHPWDSGADGGTSVQAEASLPRNLLFKYATNSE
EVIGVMSKEYIPKGTRFGPLIGEIYTNDTVPKNANRKYFWRIYSRGELHHFIDGFNEEKS
NWMRYVNPAHSPREQNLAACQNGMNIYFYTIKPIPANQELLVWYCRDFAERLHYPYPGEL
TMMNLTQTQSSLKQPSTEKNELCPKNVPKREYSVKEILKLDSNPSKGKDLYRSNISPLTS
EKDLDDFRRRGSPEMPFYPRVVYPIRAPLPEDFLKASLAYGIERPTYITRSPIPSSTTPS
PSARSSPDQSLKSSSPHSSPGNTVSPVGPGSQEHRDSYAYLNASYGTEGLGSYPGYAPLP
HLPPAFIPSYNAHYPKFLLPPYGMNCNGLSAVSSMNGINNFGLFPRLCPVYSNLLGGGSL
PHPMLNPTSLPSSLPSDGARRLLQPEHPREVLVPAPHSAFSFTGAAASMKDKACSPTSGS
PTAGTAATAEHVVQPKATSAAMAAPSSDEAMNLIKNKRNMTGYKTLPYPLKKQNGKIKYE
CNVCAKTFGQLSNLKVHLRVHSGERPFKCQTCNKGFTQLAHLQKHYLVHTGEKPHECQVC
HKRFSSTSNLKTHLRLHSGEKPYQCKVCPAKFTQFVHLKLHKRLHTRERPHKCSQCHKNY
IHLCSLKVHLKGNCAAAPAPGLPLEDLTRINEEIEKFDISDNADRLEDVEDDISVISVVE
KEILAVVRKEKEETGLKVSLQRNMGNGLLSSGCSLYESSDLPLMKLPPSNPLPLVPVKVK
QETVEPMDP

FIGURE 16

GGGGGAAGAGTAGTCAGTCGCTCGCTCACTCGCTCGCTCGCACAGACACTGCTGCAGTGACACTCGGCCCTCC
AGTGTCGCGGAGACGCAAGAGCAGCGGCAGCACCTGTCCGCCCGGAGCGAGCCCGGCCCGCGGCCGTAGAAA
AGGAGGGACCGCCGAGGTGCGCGTCAGTACTGCTCAGCCCGGCAGGGACGCGGGAGGATGTGGACTCGGTGGA
CATGAGAGAGGCTTATCTCAGATGTTGGATCTTCTCTTGGAAAAACGTGTGGGTACGACCTTGgtaaggaacc
agattctgtctttaatacgatttgaaacctttatccctttttctttcttcctttttttttttaacttttcttt
tctcccccctcccccttttaaaaaaaaaaaagaatgaagcctcagtagaaaccagcgcttctgttttagtac
gcggagcactgtcaaacatttagaagactttttcctccgtatgaatcattaaccctttcagttctagacata
attgtcaattcactgaaatttcagtagtggttcttgtccgcttcgccactcgctgcctttacattactgtaac
tatcccgggttgacttaggttttcacttgtatttaacatcgtttgttccacatggaccttacatgttggaact
aaataagaatgagatagtttaagttgtacccgggacaaggacaagtaagcatctttccccttctcggagcgtc
ctatctagggacgaattgtaaagaccagctccggagagggactcccgctgtactgtgtttacattttcacaag
cgcgcgttctaacatggttatccttattcctaatttttatctgcggcgtctatgtgggaatacgttgcagagg
ctgttttatctttcttgcttttcctctttggaaaggactttttccgagggcagataagaggaggatccccaag
tcttctgtataactttagttacagtaaactgtgccacttcagtgacttctgggaattcatgcactttcacatt
taaatagaaagtgctatttgtggctgagggctcctaaaggaattctcttcagggaattctattgactttttt
ttaatatgttttgtttttaatttttctatctggctcgagatgcccacggattaaaaaaacaaacaaaactgct
gggtgtttccctcttccccaatttttcttttcctgtggtccatgggagctcgggaaggctggtactcaaggat
gctggcaggatgcaacccctctcaggcttgcctgctggaggatgaaagagactgaacgcgcgcgcgcgcgcgg
cagagggaggggacctagttgttttgaaagttgcttcgctagggagctggtgggaaagttcagttttccccat
ttggaaaaggcagactgggttccgctcctgcaccacacgtgttttccatttttagcttcattcagacgcaggc
agcgcccctgcctcttcctccccttgtttgtgacacttctctgagacagcttttccacagctctgagggtctg
gcggccatgaccccgggcgtcccgggacacaggacgcagcagcgcccacaacacatttctgccttgagtgata
aagccaaggattgttcaaaggtagctgttctttctctcccgatgaggttaacatatacatatacgctttttt
tttttttcag**CCAAAGGCTGCATTTTAAAACCGTGCTTCTTCAAGGCAGTCTACTTTACACGGCTTTGGACT**
CTTACTCAACTGTACAAgtactccaagcttttaaagtcttcagagcaccgtgttagtcatagcctctaagagg
gaggcacaggagcgccggacaatggggattaaagcctttcccttctcttccag**GCTGCCCCCAAGTCTAGCT**
CCGGCTCCGTGAAGTTTCAAGGACTGGCAGAGACTGGGATCATGAAAATGGACATGGAGGACGCTGATATGAC
TTTGTGGACAGAGGCCGAGTTTGAAGAGAAGTGTACATACATAGTGAACGACCACCCCTGGGATTCCGGCGCT
GACGGGGGTACTTCTGTTCAAGCCGAGGCATCCTTACCAAGGAACCTGCTTTTCAAGTATGCTGCCAACAACA
GCAAAGAGgtaagccggctgccttcttgaagtctgactggcaattgggccagctctcctactactatctctga
gaaccgtgagaatttatatgcattggcaaataattgatcgctccagtggctgttttccttgctttctcttcaa
accaattcctattcatttcttcctcccttcagctgtcctatactaattagtaaacagttaaatttttggcaa
gttgacatgtcttgggaaagctaactggcagcactggtgggcagcatggtaaagggctcagtgcttcaccccc
tggccctcttggatgacagttttaaaggaaagaaacttccttagaaaaagaagtttttcctctgctcatgaga
tggctttattcttttaacgagccagctttattagctgggtttctaaaattattctcaaaaccttgacgtgttt
atgaactgaagagatggcattaaccaggaagagggtcacgtaaaagtgtcctctgtcaggatgacttcactaa
ccacccctttacctgtggcagctccctggcctgggccaggccggcaggtccatgttttatggcttctgaagtgg
gtacactctttgtatcaaagacacagaacacctgaggagcacctgatttgtgtttatataacaattagagtcg
gctgtgaagtgatttgcaaaataactccttgctctgagaatctggctgctgcagttgctctcctgatggctta
agttgctgaggctagccctgaggagacttcccaccatcaccattgcccacagtgctgtggtttctgatccttg
ctgtctttggggagnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnn
nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnaaggagggattctgtgttgtaaggattccagatttgctccta
ggactatgcattatgaaagtgccaggtcactttcctccttccctaagcgacaacatggngaaagaaactccag
agttggggaaacactgtctctttcctgtttgtgtgtctcagttcagggtttgggggggaaggagggaggtgg
caaagaccgagagctaaacttaaagagaggaattctaccttgtgtttcactaaagctccactctttgttctat
ttctgatgggagacttatttgttttatgaaaagcaaattttaaattgtagtttgtgtataaataccaccaaaa
aaaattgtaaccccagaggtcacccttaattatacctctttctgaaaacaatgttcttctcaacagaaagcct
ctgagtatgtaacccgtggagtcaatgtggcgtttgtttgggtatttgaggtctggctgtggctttaaaggt
gtagtgtgttctccccccatcagatttgcctaaatattggtcccttcaaaaagggccattcatgtcctgttgt
ctctgtagcctgttgggatagaaataaagactcctggtttttgtcatcggctctgttctccttcagagtcct

FIGURE 17

tctcagctcagccctataaagtttttcaatttttgttgttctactgaaaactatggctgaacggagttatgct
attagatcatttagttcaaggttcagatagtcaacttccccgaaggctagagttacagccaggcccagactgg
gagcaaaagttctctgattgctccaggtgtttctagatcatgctactctggtcccctcttggtgtcactgtggct
ttatttatcttagcataattcaggcacagcttttcactaagcctgtttgattctgtttcttttctgctgaatt
ccttcccatctctgtatgttaagcaaccagaaagaggcagctcttgtgtttctgacagggtgcccttcacaac
acacagaaagtcagggttctttgagcctactctatgttttaccgaaagaaaagtcaaatcgttgtggtagcac
ctcttcggtcctcaatcatgaaagccaccaggaaacaaaatttaagcatcatcctatcctaagcatacaccaa
aatgtgggcctgtttttcccttctctccagcgccttctgttttgaacttgggacaggtcccttggggtggtg
aaagagaaggacagaattgagccattgagggtgtgatagctgcaaaccagttccaggccagaagcatctagga
tggaggaagtgtttacattaccattgttcagttagttcggtgagttccatgagctgtagaaacagggaaaggc
ctttctacgggtagcttttatctgactgatctggcttgcccatctttaaatgttttcattaaaatagcaaaag
ctgttactttatgtactgtgtgctgacatcctggggacttttttttaaaaaaaaaaaaaaacaagatcatttt
tcattatgaatttgtgctatgtgtcagcaccttcccaccccacccccacccccttgatttaaaaacaaaaca
tgcaaactgtatttctgatcttaacatcaatttttagctctggctcagaatcattctgttcttttattggggc
cgtttgatcattcttacttcctatttccagatctaaaaccaagtacaagcacactcttcaaaacttttagaac
gtttaaccgggtgggttcttcttccgtgggatttcccccctcgtcctcctgaaataatctctgaatatttgact
gcattaaaaaaaaatcagttttgacattgggagagcagtatccaggacaactttctgctgtcagctgaggtgg
cttgtcctcaggcactgtgtggctcctctgactctggtctgaagttgagtggtgacaaacatcaggcagatct
agggacatggtgtccattctgattgaagtccattattaaattgagccatctgtatggaatctcagcacaacat
gcaattccacctccggacttttttatttggtagtatgtgccagagtgccaccacatctcccttcacataaaga
taacacgaagcaggagacctttcaaaaacacctgctaattcctttgaaaactggactgaagtacagacttgat
atctcggtctatacccttatagtaaaaatacaggaaaggaaatctaattgttgggcgagatttggggagggga
aaaacagcaagtaattaaagtaaccttaatttgaagtgaggaaactcaggccacttttggaactgaatagtgt
ctcctttcaaatccccctgctttctactgggctcctatatcgaacactctctaaatggctgtttgttttgaaa
gcaaacaaacagacctatgctgcccttatcatccgactttcattctgtactcccactcccactcactccatcc
tgcactgtaggaatctttctttttctaaagttaaaaagaagctctgctttttgtctctgagctccagtttgtgt
cctatagccaaagccactgacagagacagatgtgcccaggtggcagaggggtcgggctccgcaagtcaagcaag
ccatgaagagcttccgtggggctgaagcatgggcagcctaaggaggcctctttctcttcctgatgggtgtagg
gaaggcacacagcagccagtccccaggagtaattctttaggaccctggtcagttccagtgaagtttctttcct
cagttaggtgagcacactcttggtctttttagcagtgtgcaactcaaagcacaggtcaagtttctctgtgccc
tctgcttgttcctagggtagggcactgtaaacaatgggttaggaagatgggttgggctaagacactcatctgg
atcagtttcctgctttccattaagaacaaccctggctcttaccccatcctaagacaccccacaatcaccata
accttagagccgcagtttctatgtcttctttgattaaggaatcagacatggattagacgtgaaggtgtttatt
gtgtgttgttagcatgctgaacttgagatggctataggtggtttatttgatgttctttttttctgagatagaat
cttgagatgtctgtagtcggaatgcaggccacaagctgctgatcttcctgcatgctatgattagaggcatgca
ccacaccacacctagtgagacaggtgatttcgaagctacagggttcctctaacctcagaatgctgaattgtgt
gtctctgtttacctatcagtgtgaatttcctcgtgttcttcagctctgtgtatagtaggaattttaaatatca
aggtcctattgtactcataaaaacaatctaatattgcattttggtctggttatatttcccagactgaccttgg
actcaataatctcttgcctctgcctcagagtggctgggacttgaagccaccccagaatttctggttactcctt
tttctctcattcacatgacttgtctgttgagttgcatttgtaaggttaaagatggcaggcgctccaaacactg
acttaaacagctacagtgccaactccatagttgtgcctaaaaggtcctagatagcttgtaccttctttctttt
tctccccccccccccccccaagattctaaataacttcctttggccacaggacaatgtgttttgttagcgtta
aaatcggtaggtgaaaaccaagcgtctcttctgtagagagaatggcaatctggaagggaagctgtgacctcat
tgtactgctccttgttggtaatcaagctctgactcccaagaaactgtgcgcaggaggctataatttaaaacaa
agttgatgaaccagtcgagtgccttttcttaaaattactgctttaaagtggatatgttgaaattatcagctgct
aattattggctccctgacaaatggcattatttgtttttcctgcttggcattttaatattatggaataagcatt
caaatgtaaatgtctaataatttgtgtattatagaagacaattccatggattttacagagtgggttcaataat
tcacccgaacaagcctgggaccggaaagtgtagtcaagcattctgtgtaaaaatttatctccagagtctctgc
tctgagatactcttgttcccccaaagctaggctaccagcagacaccaccaatgaggaggtgtcttggaagcat

FIGURE 17 cont.

agacaggtgtgacgagggcagaggaggagacgaacatctgctttcttcagacctttgccacaaattcaaacag
gaccaaaaataacaccttaactcaacgaatctgtttcgaattgtgctggatatagagaatttctcctctccc
ctttcctgtcttaagaatgccctgttaacatgtattgaatattaaattatttaacaaaatggcatctgacgat
aaaaggacacctgtcatgagagtggcctgtccccactcttcatgtgtgtttattccacactagacccgagttt
taactatggatgaggctggataccagtgtgtatcacagtccaagtctcagatttccactcaaactacctgaag
agacattgatttgcttctctgcacctaccattcaggaatataatcataggagaaccgtcaacaaaaaaagcca
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FIGURE 17 cont.

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FIGURE 17 cont.

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FIGURE 17 cont.

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ACTACCAGAGAGCAGAGCACCTCAGTGCAGAGGTGAGGGTGTGTAGCATCTTGCGATGGATAGAAACACCACA
CCATCCAGTCGCATTT

FIGURE 17 cont.

GGGAAGCCAGACGGTTAACACAGACAAAGTGCTGCCGTGACACTCGGCCCTCCAGTGTTGCGGAGAGGCAACA
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FIGURE 18

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FIGURE 18 cont.

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FIGURE 18 cont.

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FIGURE 18 cont.

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tattacttgaaatgagtcttaatctttcacatttagttctcagggtatgctgatttcctttaggtaaaccatg
aacatcagaaagacttttattaacctatgacagggtccccaccccagtatttttccactccattaaaatggaa

FIGURE 18 cont.

gttttttttttttttttctttttgagacagagttttgctcttgttgcccagtctggagtgcaatggcacaat
ctcggctcaccacaacctccacctcccagattcaagcgattcttctgcctcagcctcccaagtagctgggatt
acaggtgtgccaccacgcccagctaatttttgtatttttagtagagatggggtttctccatgttggtcaggc
tggtctcgaacttccgacctcaggtgatccgcccacctcggcctcccaaagtgctgggattacaggcaagagc
cactgcatccagcttaggctatcttactccagcctaaacagcaatttttctatcataaggtctgtactaatgaa
aacagaatcacccaaggctgctgtttgttctgtctgtgctgccattgtccgcattttgctgaggaggaaacgg
aactgcacttttgagtgagtggcccagagccttctagaatgagagtgcgttggaagccagatatgtggcgatt
gtgtcgccagctgttactcaggtttctcaagaaggaggagcaactttggcagttttgcttcagttctctcta
gccctctgtgtaatcgcccctttttctttatttcagCACAAACACAGAGCAGTCTAAAGCAACCCGAGCACTGA
GAAAAATGAACTCTGCCCAAAGAATGTCCCAAAGAGAGAGTACAGCGTGAAAGAAATCCTAAAATTGGACTCC
AACCCCTCCAAAGGAAAGGACCTCTACCGTTCTAACATTTCACCCCTCACATCAGAAAAGGACCTCCATGACT
TTAGAAGACGTGGGAGCCCCGAAATGCCCTTCTACCCTCGGGTCGTTTACCCCATCCGGGCCCCTCTGCCAGA
AGACTTTTTGAAAGCTTCCCTGGCCTACGGGATCGAGAGACCCACGTAGATCACTCGCTCCCCCATTCCATCC
TCCACCACTCCAAGCCCCTCTGCAAGAAGCAGCCCCGACCAAAGCCTCAAGAGCTCCAGCCCTCACAGCAGCC
CTGGGAATACGGTGTCCCCTGTGGGCCCCGGCTCTCAAGAGCACCGGGACTCCTACGCTTACTTGAACGCGTC
CTACGGCACGGAAGGTTTGGGCTCCTACCGTGGCTACGCACCCCTGCCCCACCTCCCGCCAGCTTTCATCCCC
TCGTACAACGCTCACTACCCCAAGTTCCTCTTGCCCCCCTACGGCATGAATTGTAATGGCCTGAGCGCTGTGA
GCAGCATGAATGGCATCAACAACTTTGGCCTCTTCCCGAGGCTGTGCCCTGTCTACAGCAATCTCCTCGGTGG
GGGCAGCCTGCCCCACCCCATGCTCAACCCCACTTCTCTCCCGAGCTCGCTGCCCTCAGATGGAGCCCGGAGG
TTGCTCCAGCCGGAGCATCCCAGGGAGGTGCTTGTCCCGGCGCCCCACAGTGCCTTCTCCTTTACCGGGGCCG
CCGCCAGCATGAAGGACAAGGCGTGTAGCCCCACAAGCGGGTCTCCCACGGCGGGGAACAGCCGCCACGGCAGA
ACATGTGGTGCAGCCCAAAGCTACCTCAGCAGCGATGGCAGCCCCCAGCAGCGACGAAGCCATGAATCTCATT
AAAAACAAAAGAAACATGACCGGCTACAAGACCCTTCCCTACCCGCTGAAGAAGCAGAACGGCAAGATCAAGT
ACGAATGCAACGTTTGCGCCAAGACTTTCGGCCAGCTCTCCAATCTGAAGgtaggccttgagagagagcagtc
caaggggctgtgagtgcatgcttgtgtttgtatttagcttgctttccatggggtatcgattgcatttgcagta
gtatgagccccggttggggatagtgggtatggattccgcctggcttttgccacttctagctctttgactttg
gacaagtgacttcccttctcctgatttttcttctgaataataaaaaaattaggggtttggactagaagattagg
tgaaactccctgctagcctgtgatttttgtgcttttaagaaaaacaccattctgaaaacatgaagatttcttc
ttttaagactgtcttgatgcttttcttaagatatttgcatcaacacttgagtcttggagcagaaatgttagg
tctcagagccagcttgagagcagagctaacacatgtggcttcttcccagGTCCACCTGAGAGTCCACAGTGGA
GAACGGCCTTTCAAATGTCAGACTTGCAACAAGGGCTTTACTCAGCTCGCCCACCTGCAGAAACACTACCTGG
TACACACGGGAGAAAAGCCACATGAATGCCAGgtgcgcagtattttctgggtagaccttctgaccttttgtaga
aaatgtctgtgagtcaccctcccatgtcctatatagcccgtagttaaagccaacaccagattctgcgttgtcc
catcctggactgatggcactatggtccttcccagtactttgtatctgctgatgacttgagatggcacagccag
cttccagtgggtgggaaaatggtagggggaaataaacagccctcgtgtgctgtgtgcccacatccccccgttt
gcttaataccacactggaggtgccacaaggaggcttctcacctcctaggttgctgggcgttggccggtaagcc
tgccctcccgttggcaactcttaatcttctggccttcctgtctcccttcccctgctgtctctctccccctacac
tgtagGTCTGCCACAAGAGATTTAGCAGCACCAGCAATCTCAAGACCCACCTGCGACTCCATTCTGGAGAGAA
ACCATACCAATGCAAGGTGTGCCCTCCCAAGTTCACCCAGTTTGTGCACCTGAAACTGCACAAGCGTCTGCAC
ACCCGGGAGCGGCCCCACAAGTGCTCCCAGTGCCACAAGAACTACATCCATCTCTGTAGCCTCAAGGTTCACC
TGAAAGGGAACTGCGCTGCGGCCCCGGCGCCTGGGCTGCCCTTGGAAGATGTGACCCGAATCAATGAAGAAAT
CGAGAAGTTTGACATCAGTGACAATGCTGACCGGCTCGAGGACGTGGAGGATGACATCAGTGTGATCTCTGTA
GTGGAGAAGGAAATTCTGGCCGTGGTCAGAAAAGAGAAAGAAGAAACTGGCCTGAAAGTGTCTTTGCAAAGAA
ACATGGGGAATGGACTCCTCTCCTCAGGGTGCAGCCTTTATGAGTCATCAGATCTACCCCTCATGAAGTTGCC
TCCCAGCAACCCACTACCTCTGGTACCTGTAAAGGTCAAACAAGAAACAGTTGAACCAATGGATCCTTAAGAT
TTTCAGAAAACACTTATTTTGTTTCTTAAGTTATGACTTGGTGAGTCAGGGTGCCTGTAGGAAGTGGCTTGTA
CATAATCCCAGCTCTGCAAAGCTCTCTCGACAGCAAATGGTTTCCCCTCACCTCTGGAATTAAAGAAGGAACT
CCAAAGTTACTGAAATCTCAGGGCATGAACAAGGCAAAGGCCATATATATATATATATATATCTGTATACA
TATTATATATACTTATTTACACCTGTGTCTATATATTTGCCCCTGTGTATTTTGAATATTTGTGTGGACATGT

FIGURE 18 cont.

TTGCATAGCCTTCCCATTACTAAGACTATTACCTAGTCATAATTATTTTTTCAATGATAATCCTTCATAATTT
ATTATACAATTTATCATTCAGAAAGCAATAATTAAAAAAGTTTACAATGACTGGAAAGATTCCTTGTAATTTG
AGTATAAATGTATTTTTGTCTTCTGGCCATTCTTTGTAGATAATTTCTGCACATCTGTATAAGTACCTAAGAT
TTAGTTAAACAAATATATGACTTCAGTCAACCTCTCTCTCTAATAATGGTTTGAAAATGAGGTTTGGGTAATT
GCCAATGTTGGACAGTTGATGTGTTCATTCCTGGGATCCTATCATTTGAACAGCATTGTACATAACTTGGGGG
TATGTGTGCAGGATTACCCAAGAATAACTTAAGTAGAAGAAACAAGAAAGGGAATCTTGTATATTTTTGTTGA
TAGTTCATGTTTTTCCCCCAGCCACAATTTTACCGGAAGGGTGACAGGAAGGCTTTACCAACCTGTCTCTCCC
TCCAAAAGAGCAGAATCCTCCCACCGCCCTGCCCTCCCCACCGAGTCCTGTGGCCATTCAGAGCGGCCACATG
ACTTTTGCATCCATTGTATTATCAGAAAATGTGAAGAAGAAAAAAATGCCATGTTTTAAAACCACTGCGAAAA
TTTCCCCAAAGCATAGGTGGCTTTGTGTGTGTGCGATTTCGGGGCTTGAGTCTGGGTGGTGTTTTGTTGTTGG
TTTTTGTTGCTTTTTTTTTTTTTTTTTTTTTTAATGTCAAAATTGCACAAACATGGTGCTCTACCAGGAAGGAT
TCGAGGTAGATAGGCTCAGCCACACTTTAAAAACAAACACACAAACAACAAAAAACGGGTATTCTAGTCATC
TTGGGGTAAAAGCGGGTAATGAACATTCCTATCCCCAACACATCAATTGTATTTTTTCTGTAAAACTCAGATT
TTCCTCAGTATTTGTGTTTTTACATTTTATGGTTAATTTAATGGAAGATGAAAGGGCATTGCAAAGTTGTTCA
ACAACAGTTACCTCATTGAGTGTGTCCAGTAGTGCAGGAAATGATGTCTTATCTAATGATTTGCTTCTCTAGA
GGAGAAACCGAGTAAATGTGCTCCAGCAAGATAGACTTTGTGTTATTCTATCTTTTATTCTGCTAAGCCCAAA
GATTACATGTTGGTCTTCAAAGTGTAGCAAAAAATGATGTATATTTATAAATCTATTTATACCACTATATCAT
ATGTATATATATTTATAACCACTTAAATTGTGAGCCAAGCCATGTAAAAGATCTACTTTTTCTAAGGGCAAAA
AAAAAAAAAAAAAAAAAAGAACACTCCTTTCTGAGACTTTGCTTAATACTTGGTGACCTCACAATCACGTCGG
TATGATTGGGCACCCTTGCCTACTGTAAGAGACCCTAAAACCTTGGTGCAGTGGTGCGGACCACAAAACAACC
AGGGAGGAAGAGATACATCATTTTTTAGTATTAAGGACCATCTAAGACAGCTCTATTTTTTTTTTGCCACTTT
ATGATTATGTGGTCACACCCAAGTCACAGAAATAAAAAACTGACTTTACCGCTGCAATTTTTCTGTTTTCCTC
CTTACTAAATACTGATACATTACTCCAATCTATTTTATAATTATATTTGACATTTTGTTCACATCAACTAATG
TTCACCTGTAGAAGAGAACAAATTTCGAATAATCCAGGGAAACCCAAGAGCCTTACTGGTCTTCTGTAACTTC
CAAGACTGACAGCTTTTTATGTATCAGTGTTTGATAAACACAGTCCTTAACTGAAGGTAAACCAAAGCATCAC
GTTGACATTAGACCAAATACTTTTGATTCCCAACTACTCGTTTGTTCTTTTTCTCCTTTTGTGCTTTCCCATA
GTGAGAATTTTTATAAAGACTTCTTGCTTCTCTCACCATCCATCCTTCTCTTTTCTGCCTCTTACATGTGAAT
GTTGAGCCCACAATCAACAGTGGTTTTATTTTTCCTCTACTCAAAGTTAAAACTGACCAAAGTTACTGGCTT
TTTACTTTGCTAGAACAACAAACTATCTTATGTTTACATACTGGTTTACAATGTTATTTATGTGCAAATTGTC
AAAATGTAAATTAAATATAAATGTTCATGCTTTACC

FIGURE 18 cont.